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Atomic Spectrometry Update: review of advances in elemental speciation

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Abstract

This is the 12th Atomic Spectrometry Update (ASU) to focus on advances in elemental speciation and covers a period of approximately 12 months from December 2018. This ASU review deals with all aspects of the analytical atomic spectrometry speciation methods developed for: the determination of oxidation states; organometallic compounds; coordination compounds; metal and heteroatom-containing biomolecules, including metalloproteins, proteins, peptides and amino acids; and the use of metal-tagging to facilitate detection via atomic spectrometry. As with all ASU reviews the focus of the research reviewed includes those methods that incorporate atomic spectrometry as the measurement technique. However, because speciation analysis is inherently focused on the relationship between the metal(loid) atom and the organic moiety it is bound to, or incorporated within, atomic spectrometry alone cannot be the sole analytical approach of interest. For this reason molecular detection techniques are also included where they have provided a complementary approach to speciation analysis. This year the number of publications concerning As speciation has fallen by about half as have those for studies on Se speciation. Growth areas continue to be Hg and 'biomolecules', with the number of reports concerning halogen and sulfur speciation also rising. The number of elements covered this year is again over 20 showing the breadth of the elemental speciation field.

Contents

1 Topical Reviews

This latest update adds to that from last year[1] and complements the five other annual Atomic Spectrometry Updates, advances in environmental analysis[2], advances in the analysis of clinical and biological materials, foods and beverages[3], advances in atomic spectrometry and related techniques[4], advances in X-ray fluorescence spectrometry and its special applications[5] and advances in the analysis of metals, chemicals and materials.[6]

Two *books have appeared in the most recent review* period containing chapters dealing with elemental speciation topics. *The Handbook of Smart Materials in Analytical Chemistry* is a two volume set dealing, in volume 1, with the various types of smart material, and, in volume 2, the analytical processes (part 1) and analytical applications (part 2) of materials that are "tailored, task-specific, or designed . . . to provide tremendous enhancements of practical properties, such as selectivity, sensitivity, easy automation or speediness." The first volume consists of 15 chapters. Part 1 of volume 2 consists of seven chapters, five of which are devoted to an analytical procedure (SPE, SPME chromatography, CE, immunoassay, and LDI-MS) and three to broader topics (miniaturization in sample handling and preparation, immunoassays, and trace element determination/speciation). This latter chapter consists of several sections (19 pages) devoted to various sorts of nanomaterials, a section on ion-imprinted polymers and several subsections devoted to speciation of the following elements: As and Sb, Cr, Hg, Se, Sn, Tl and V.[7] Speciation of a subset of these elements (As, Cr, Hg) by "sensing probes" takes up a further 8 pages. Part 2 of the second volume consists of chapters devoted to the analysis of particular sample types (water, food, clinical, pharmaceutical and forensics), but it is not clear whether any elemental speciation topics are included in these sections. Overall the two volumes are about 1,000 pages long. In *Sediments: Chemistry and Toxicity of In-Place Pollutants*, the third chapter is devoted to "Inorganic Sediment Chemistry and Elemental Speciation" and includes a section on speciation methods and examples of their application to river sediments.[8] A later chapter deals with the biomethylation and cycling of certain metals and metalloids in aquatic sediments.[9] The elements include As, Hg, Pb and demethylation and abiotic mechanisms are also discussed along with bioaccumulation/bioconcentration in aquatic organisms.

Clearly, any work on the *coupling of HPLC instrumentation with ICP-MS* detection has application in speciation analysis. The performance of 31 combinations of commercially available nebulisers and spray chambers for the introduction of organic solvents has been evaluated in terms of the contribution to peak broadening.[10] This is part 2 of a series, for which part 1[11] was a theoretical study of extra-column broadening and a critical examination of the results of a number of published experiments. The Part 2 experiment consisted of injecting 1 µL of a V porphyrin compound into an acetonitrile carrier stream flowing at 400 µL min^{-1} that passed first through a UV detector and then into the ICP-MS. Peak broadening was calculated as variance in units of μL^2. Amazingly, values ranged from 10 to 8,000. The researchers discussed the reasons for the performance of several particular nebuliser / spray chamber combinations in some detail. In addition, the effects of spray chamber temperature and flow rate are discussed. The term “direct injection” does not appear in the article, though one of the spray chambers is described as “total consumption.” Flow splitting (not a good idea according to the researchers) was considered in part 1. The suppliers of all the nebulizers are identified, but not for all of the spray chambers. Recommendations were made for the most suitable combinations, which were somewhat limited in the case of UHPLC.

The current situation regarding the *detection of engineered nanoparticles in aquatic environments* has been reviewed (212 references)[12]. The reviewers focus on online or *in situ* analyses by enrichment-separation-detection. Enrichment methods covered include coagulation, flotation, filtration (membrane processes), followed by microfluidic separation and sorting. Finally, determination in terms of size, concentration, and species is discussed. The review includes methods involving a range of instrumental techniques, including ICP-MS, which is featured in a separate section devoted to the contents of some 11 original articles, as well as being mentioned throughout the review. Single-particle ICP-MS (sNP-ICP-MS) is also discussed, though not in any great detail. There are several references to speciation analysis, for which ICP-MS is the detector. The reviewers concluded that there is not yet sufficient information to allow the real impact of engineered nanoparticles in the aquatic environment to be evaluated, and that improvements in analytical methodology are needed, particularly with regard to combined precontraction and separation procedures.

Among several reviews of the applications of particular instrumentation is the biennial survey of advances in *CE with MS detection* (248 references).[13] Only about 10% of the cited articles feature studies with ICP-MS detection, and these are divided between about 5 articles describing developments in the interface and about 18 articles describing applications to particular sample types, including food, environmental, actinides, NPs (all Au), pharmaceutical and clinical. The reviewers consider that although CE with ICP-MS detection is now routinely applied for (trace) elemental analysis and commercial interfaces are available, there is still room for improvement, especially with regard to the interface design. They identify three types of CE-ICP-MS interfaces: sheath flow, sheathless, and something called the “hybrid generation” interface. Of these, the sheath flow interface is most frequently used. The bulk of the review concerns applications with organic mass spectrometry, predominantly for the characterisation of proteins, peptides and metabolites. Work on interfacing with MALDI is included, though the bulk of the development work concerns nano-ES. Various modes of CE separation are also covered, including nonaqueous CZE, capillary gel electrophoresis, and cIEF.

Two reviews of different aspects of *VG for atomic spectrometry* touch on speciation. Though this is very brief in a review entitled modern chemical and photochemical vapour generators for use in OES and MS, in which maybe two of the 113 references describe elemental speciation work.[14] The focus of the review is on the performance of systems by which both vapour-forming elements and non-vapour-forming elements can be introduced to the spectrometer simultaneously, thereby exploiting the additional detection capability that arises from the increased sample introduction efficiency for the vapour-forming elements. Much of the work included is summarised in two tables: one for multi-modal introduction and the other for photochemical vapour generation. There is no mention of electrochemical generation or generation from solid-phase reagents. In the conclusion, further developments are envisaged, including that of a multi-channel sample introduction system consisting of a multichannel ultrasonic nebulizer, or a set of pneumatic nebulizers, together with a heated spray chamber, that would enable chemical/photochemical vapour generation with on-line calibration (including internal standardization). The second review (96 references, with titles) summarises the developments and applications of "nanomaterials/enhancement reagents" used in photo-assisted VG for analytical atomic spectrometry.[15] Such materials include TiO_2 and TiO_2-based composites, other metal oxide framework compounds and metal ions. The review also covers nanomaterials for preconcentration and separation. About 20% of the articles cited have "speciation" in the title, though these are not covered in any coherent fashion and are sprinkled throughout the review. The reviewers highlight four areas for further research: (1) more kinds and combinations of photocatalysts to improve generation efficiency (which might lead to more elements, such as Cr, Re, Ru, Tc, Tl and W and Re. being vapourisable); (2) elucidation of mechanisms; (3) applications to indirect determination of biomolecules and samples of with complicated matrices; and (4) the development of miniaturized generators for introduction into micro-plasmas, which are unable to tolerate the large volumes of hydrogen that typically accompany conventional HG. This latter area was thought to be of considerable potential for "field" and "green" analytical methodologies, especially for the environmental determination of Hg and other toxic metals.

Elemental speciation is mentioned, though only briefly, in a review (48 references) of the combination of *elemental detection with liquid-phase microextraction* (LPME), including, single-drop ME, hollow-fibre ME, and dispersive liquid-phase ME (DLLME).[16] The focus of the review is on the possibilities for the preconcentration and/or separation of analytes from potentially interfering matrix components. The reviewers conclude that although substantial progress in the development of LPME procedures has been made, particularly with regard to new modalities, magnetic materials, and automation, the potential for the determination of metals has yet to be fully exploited. They consider the acceptance of the procedures in routine analytical laboratories depends on automation and integration with analytical instrumentation and predict that this will be realised in the near future.

The rather broad topic of *speciation analysis of environmental and biological samples for trace As, Hg, Sb and Se* has been reviewed (186 references).[17] No real justification for the review is given, and although eight previous relevant reviews are cited, there is only one citation to an Atomic Spectrometry Updates (in support of the claim than AFS is the most widely used of the optical atomic spectrometries for LC detection). The review starts with a brief section on non-chromatographic procedures, including selective extraction (30 references) and selective reduction (seven references), by which the authors mean chemical and photochemical vapour generation methods. The bulk of the review is devoted to the combination of chromatographic separation with element-specific detection, covering both GC separation (10 references) and LC (52 references). Although both ion-exchange and ion-pair methods are cited, there is no critical comparison. Nor is there any discussion of compound-dependent responses and how to deal with them. The drawbacks of gradient elution and of the limitations on mobile phase composition for ICP-MS detection are pointed out, and the possible advantages for VG interfacing discussed. The disadvantage of the dilution inherent in LC methods is also pointed out, and the reviewers consider that in some cases, if sample volume is not a limitation, a non-chromatographic procedure, that might well involve preconcentration, could be a better choice. They also consider that ICP-MS is too expensive for many routine analytical laboratories and that HPLC-HG-AFS is very promising with "vast potential" for further development.

Reviews of aspects of *the role of atomic spectrometry (mainly ICP-MS) in various bioanalytical fields* have appeared. The application of ICP-MS in proteomics has been reviewed (62 references).[18] The reviewers perhaps oversell the technique when they write that "the elemental signal . . . is independent of its chemical environment." But are correct in pointing out the considerable potential of the technique for the screening, mapping and quantification of peptides and proteins separated by chromatography, although they are rather harsh in blaming ICP-MS for shortcomings in separation methodologies. The reviewers identify the ability to quantify S as the breakthrough development, which was helped enormously by the advent of tandem MS instruments that allowed the detection of S, at m/z 48, as $^{32}S^{16}O^+$. The reviewers touch on the possibilities of applications (a) in standards certification and, (b) in combination with LA, for imaging. They critically evaluate the applications in immunoassays via metal tags and NPs, which is highlighted as a promising area for future developments. Linscheid has reviewed the information available from ICP-MS used in collaboration with MALDI-MS and ES-MS (137 references), with particular reference to the contribution of his own research group at the Humboldt University of Berlin.[19] The reviewer refers to this as the "introduction of ICP-MS to the bioanalytical workflow." Their fundamental idea was to measure elements, which started by replacing radioactive ^{32}P with the "cold" natural ^{31}P to quantify modified nucleotides, phosphorylated peptides and proteins. Later, they developed tagging strategies, based on the complexes of lanthanoids with DOTA (1,4,7,10-tetraazacyclododecane-N,N ',N '',N"'-tetraacetic acid) for the more general quantification of proteins using multi-isotopic elements and accurate ID absolute measurements. The tags provide low LOD values and offer multiplexing capabilities due to the number of very similar lanthanoids and their isotopes. Also covered in the review are the combinations of separation techniques, such as electrophoresis and HPLC, with ICP-MS for the determination of nucleotides, proteins and peptide, enzyme substrates, and antibodies. Imaging MS, with both MALDI-MS and laser ablation ICP-MS, is briefly mentioned. A review (20 references) of microfluidic chip sample pre-treatment for the ICP-MS determination of trace elements and their species in cells only mentions two examples of speciation analysis (Se amino acids and inorganic and organic Hg species)[20]. At present, the reviewers consider that ICP-MS has the limitations, when applied to the analysis of cells, of (a) insufficient LOD values, (b) interferences from the complex cell matrix, and (c) incompatible sample consumption. The bulk of the review is taken up with the design and manufacture of, and the manipulations possible within, the various types of chip that have been reported, including various types of microextraction and droplet encapsulation. Examples were discussed of methodologies with high sensitivity, high throughput, good matrix tolerance and low sample and reagent consumption, contributing to the quantification of trace elements and species in cells and even in single cells. The reviewers made a number of suggestions for the direction of future research: (a) more packing materials with good selectivity, high adsorption capacity, and fast reaction kinetics, (b) the combination of microfluidic chip-ICP-MS or -HPLC-ICP-MS with other analytical techniques, such as HPLC-ES-MS/MS, and (c) the capability for single-cell analysis should be improved, which together with the further development of elemental labels for key biomarkers would facilitate studies of the uptake of metal-containing drugs or metal-containing NPs.

Several reviews of *mass spectrometry imaging* have appeared. What is described as a concise review (169 references) of applications in pharmaceutical research and development contains very little elemental MS: just three studies are mentioned under the heading of "complementary optical techniques," that involve LA-ICP-MS for imaging cytometry.[21] However, a slightly shorter review (139 references) of *in situ* metallomic imaging[22] features numerous studies that provided a picture of an emerging surge of elemental bioimaging with LA-ICP-MS as a cornerstone technique, allowing fast generation of isotopically specific, element-distribution maps of biological samples down to spatial resolutions of one µm, thereby opening applications at the cellular level. Furthermore, the reviewer considers that developments in TOF-MS, which allows simultaneous determination of almost the entire periodic table, makes LA-ICP-TOF-MS incredibly promising for the field of metallomics, as it presents the first opportunity to capture the spatial distribution of the metallome in biological samples. The review also covers a variety of X-ray techniques, including XANES and EXAFS, which are capable of providing information about elemental speciation. Despite calling element 35 bromium, the review is recommended reading. There have been enough studies involving the elemental imaging of brain samples to warrant a review (147 references) of "the growing importance" of analytical atomic spectrometry in metal imaging in neuroscience.[23] Although the term "speciation" does not appear in the review, there is considerable coverage of X-ray methods, including some applications of XANES. The possibilities of LA-ICP-MS are given considerable prominence and the development of applications is nicely illustrated in a figure compiled from several sources. Also mentioned are nano-SIMS and PIXE, and there is a section entitled "emerging methods" that largely features LA-ICP-MS, but also includes some discussion of X-ray fluorescence microscopy. There are two applications sections: one concerned with Cu and Fe in Parkinson's disease, and the other with the accumulation of metals by amyloid plaques. The reviewers point out that the most significant source of uncertainty in metal imaging is whether the picture generated is an accurate representation of normal physiology because sample preparation, storage, and the measurement itself all affect sample integrity. They suggest that multimodal imaging would not only confirm the observations made by various techniques applied on their own, but also provide additional information related to elemental speciation or isotope tracing. The also consider that imaging mass cytometry will have a major impact in the neurosciences as this technology becomes more widely applied. In a much broader review (206 references) of the imaging of protein distribution in tissues[24], elemental analysis is only a small part, as the review is confined to studies in which MS has provided the information. The reviewers highlight untargeted MS imaging of *in situ* digested proteins with MALDI as the most widely used approach, There is a brief mention of LA-ICP-MS in two of the main sections of the review, but the technique is not highlighted in the concluding remarks.

Reviews of the speciation analysis of individual elements are discussed in the relevant element sub-sections of Section 3.

2. CRMs and Metrology

A wide ranging *review of the role of ICP-MS in inorganic chemical metrology*, focussing on CCQM studies over the past 25 years has been published this year[25]. The review is wide ranging and topics covered include; the methodologies used to overcome spectral interferences, traceability and uncertainty, calibration strategies including IDMS, standard additions and high accuracy single point calibrations and a section on the application of hyphenated ICP-MS techniques which covers 11 CCQM comparisons. This latter section concludes with the observation that species identification is not always possible, due to a lack of available standard compounds and that, as there are an ever growing number of target analytes being reported in the literature, there is an ongoing need for the production of more speciated CRMs to allow this work to validated. The paper highlights this by including a list of CRMs (over 50 in number) produced by CCQM study participants which shows that CRMs certified for the content of As, Hg and Se species predominate. The paper also discusses future application areas with one section focussing on the work being undertaken to allow the quantification of proteins, traceable to the SI, on the basis of either the heteroatom content or by elemental tagging. The paper is recommended reading for anyone new to the field of chemical metrology and for established practitioners who wish to update their knowledge in this area.

The *measurement of MeHg in four existing MODAS CRMs,* M-2 BotSed, M-3 HerTis, M-4 CormTis and M-5 CodTis, by species specific IDMS has been reported on this year[26]. A spike solution of ^{201}Hg enriched MeHg was added to each sample, which varied in mass from 0.1 – 0.8 g depending on the matrix, followed by the addition of 10 mL of 15% HCl, stirring at 50 °C for 30 minutes, centrifugation, filtration of the supernatant and dilution prior to analysis by HPLC-ICP-MS. The isocratic separations were undertaken on an RP C18 column (150 × 3.9 mm, 5 µm) with a mobile phase containing 0.05% v/v 2-mercaptoethanol, 0.4% m/v L-cysteine, 0.06 mol L^{-1} ammonium acetate and 2% methanol flowing at 1 mL min^{-1}. Three Hg isotopes, 200, 201 and 202, were monitored to allow the IDMS calculations to be performed using two different reference isotopes for quality assurance purposes. Two CRMs, NRCC DORM-4 and TORT-3, were used for method validation with the found results stated to be in agreement with the certified values (although no details of any statistical test used to make this comparison is given). The main contribution to the expanded uncertainty (K = 2) of each measurement arose from the measurement of the Hg isotope amount ratios in the spiked samples and mass bias correction blend and the within bottle repeatability. The mass fraction of MeHg (as Hg) in each RM was found to be; M-2 BotSed 15.6 ± 1.0 μg kg^{-1}, M-3 HerTis 216 ± 6 μg kg^{-1}, M-4 CormTis 2106 ± 54 μg kg^{-1} and M-5 CodTis 294 ± 8 μg kg^{-1}.

In any growing analytical field *method validation protocols and metrology* tend to develop at a slower rate than the methodologies used, although this should not be the case and a metrological approach should be the starting point for method development. It is timely then that the current status of NP detection, with a focus on analytical metrology, has been reviewed.[27] The review (with 139 cited references) proposes some metrological definitions for NPs and covers the analytical methods used for both their characterisation and detection, the latter being split into two areas; methods in which NPs are used to extract and / or pre-concentrate other target analytes and methods where the NP is the analyte. After a brief section on microscopy based techniques the main focus is on the use of separation by CE, FFF and LC coupled to a variety of detectors including dynamic light scattering (DLS), multiangle light scattering (MALS), ICP-MS and UV with the use of these techniques reported over the past five years summarised in a table (60 references) many of which are also discussed in the text. The authors point out that maintaining sample integrity during both analyte characterisation studies and detection methodologies is, as for any analysis, still the key to providing analytical validity but can prove more challenging due to the tendency of NPs to aggregate and the wide range of complex matrices, from consumer products to biota, which may contain NPs. It is also pointed out that whilst NPs are available as standards, often containing a stabilising agent, with a given mass concentration, particle concentration, size or size distribution these parameters may change upon dilution (or other use) into a different matrix. A further problem highlighted is the lack of available CRMs for method validation and that, from a strict metrological point of view, recovery studies are only admissible when the other alternatives are not available.

3 Elemental Speciation Analysis

3.1 Antimony

A *review of both non-chromatographic and chromatographic procedures for the speciation of Sb* employing HG-AFS for environmental matrices have been published by Ferreira *et al.*[28] The non-chromatographic procedures were based on the determination of total Sb after a pre-reduction step and typical reductant agents used were discussed. A masking agent was often used to allow the quantification of Sb^{III}. The most commonly used reductants were citrate and 8-hydroquinoline. Chromatographic procedures utilised mainly HPLC and the advantages and drawbacks of the extracting agents and potential mobile phase composition are discussed. Improvements to the method sensitivity for the determination of Sb^{V} and its organic compounds by HPLC-HG-AFS were also evaluated as were speciation procedures involving a preconcentration step. Applications of both non-chromatographic and chromatographic procedures for speciation studies of Sb in environmental samples are also presented.

Few new *approaches for Sb speciation* have been reported in the last twelve months. A novel carboxyl-functionalized organic-inorganic hybrid monolithic column has been prepared via one-pot co-condensation of carboxyethylsilanetriol sodium salt (CES) and tetramethoxysilane (TMOS).[29] The TMOS-co-CES monolithic column was used for SPME followed by the use of ICP-MS for simultaneous speciation analysis of iCr and iSb. Various parameters used for the SPME and overall analytical performance were investigated. Under optimum conditions the TMOS-co-CES column could selectively adsorb Cr^{III} and Sb^{III} without adsorbing Cr^{V} and Sb^{V} over a pH range 4.0-9.0. Both species were eluted with 10% nitric acid (v/v). The TMOS-co-CES column operated under milder elution conditions, offered higher recovery for Sb species, and was more compatible with ICP-MS than previously reported thiol-functionalized hybrid monolithic columns. A new extraction method for the speciation of iSb in inhalable particulate matter (PM2.5) using HPLC-ICP-MS has been reported.[30] The extraction method consisted of both a reducing extraction and an oxidizing extraction using EDTA as the key component. The stability of Sb species in aqueous EDTA-saturated nitric or tartaric acid solution were investigated and under optimized conditions the method facilitated the determination of Sb^{III} and Sb^{V} in PM2.5 samples with LOD values of 0.10 and 0.06 ng m^{-3}, respectively. The RSD values were better than 8.5% for Sb^{III} and 6.6% for Sb^{V}. The spiked recovery rates of Sb species at three different concentration levels were in the range of 90-110%. The extraction yield of the process, evaluated using the NIST1648a SRM (urban particulate matter), was 60%. The method was used to determine Sb^{III} and Sb^{V} in PM2.5 samples collected in an urban area of Ningbo City, China. The results showed that both Sb^{V} and Sb^{III} were found in most of the samples analysed, with Sb^{V} being predominant at 69-87% of the total Sb extracted from the samples. A preconcentration / speciation method for Sb species in bottled mineral water samples using a $SiO_2/Al_2O_3/SnO_2$ adsorbent has been reported[31]. The method is based on the selective adsorption of Sb^{III} ions under a wide pH range (2.5-7.5). Total Sb was determined using 0.1% (w/v) L-cysteine at pH 3.5 and the concentration of Sb^{V} species was determined by the difference between the total Sb and Sb^{III} concentrations. The proposed method provided a LOD of 0.17 μg L^{-1} and a preconcentration factor of 136-fold. Recovery tests on bottled mineral water samples and the SRM NIST 1643e Trace elements in water, ranged from 95.2-106.0%. Only Sb^{III} species were determined in mineral water (0.54-1.04 μg L^{-1}).

The *determination of Sb in water samples* (sea, mineral water, tap water, or lakes) continues to attract attention. An automated flow-batch system employing HG-AFS has been reported for the determination of iSb in waters[32]. Unlike many automated flow methods that use confluent fluids and often complex devices, the main advantage of the proposed method is the use of a simple laboratory made flow-batch chamber to simultaneously perform mixing, homogenization, reactions, Sb hydride formation, and gas-liquid separation. The method was optimized using both a two-level full factorial and Box-Behnken designs and validated on the basis of repeated measurements and analysis of variance. The working range after optimisation was 100-2000 ng L^{-1} with a RSD of 4% and LOD of 6 ng L^{-1}. Recovery tests using the SRM NIST 1643e, trace elements in water, resulting in recovery rates of 90 to 114%, with a relative error of 0.7%. The sampling throughput was 54 samples h^{-1}. The determination of Sb^{III} and Sb^{V} in flavoured bottled drinking water samples using either ion exchange or size exclusion chromatography HPLC-ICP-MS and ES-MS-MS has been published[33]. The work builds on previous studies by the same group on multielement speciation and non-targeted speciation screening. This study focused on the quantification of the Sb for validation of the method, including an uncertainty budget, and screening of the different species present in the sample. No Sb^{III} was detected in any sample and Sb^{V} ranged from 0.201 ± 0.012 g L^{-1} to 0.524 ± 0.032 g L^{-1}.

There have been fewer reports of studies on *Sb speciation in food* during this review period. The determination of trace iSb in PET bottled soy sauce has been measured by IC-ICP-MS[34]. The sample was first filtered through a weak cation exchange SPE column and then subsequently washed with 3.0 mL of 0.02% L-ascorbic acid and 3.0 mL of methanol. Finally, the SPE column was eluted with 1.0 mL of 0.1% NaOH + 2% H_2O_2. The Sb^{III} on the SPE column was converted into Sb^{V} and was successfully eluted and quantified by IC-ICP-MS. The LOD for Sb^{III} was 0.1 μg L^{-1}, and the RSD (n = 6) was 8.8%. Recoveries (n = 6) were 91-97%. By comparing the amount of Sb^{III} (0.8 μg L^{-1} - 2.3 μg L^{-1}) with the total Sb (1.7 μg L^{-1} - 6.6 μg L^{-1}), 25.6% - 50.0% of the total Sb in the soy sauce was found as

Sb^{III}. The speciation of Sb in paired soil-rice samples around Xikuangshan, China, the world largest active Sb mining region, has been reported[35]. The soil and rice samples were analysed by using wet chemistry, LA-ICP-MS, μ-SR-XRF and μ-XANES spectroscopy. The results of field survey work indicated that the paddy soil in the region was co-polluted by Sb (5.91-322 mg kg^{-1}) and As (0.01-57.21 mg kg^{-1}). Despite the higher Sb concentration in the soil, rice accumulated more As than Sb indicating the higher phytoavailability of As. The Sb present was distributed similarly to As, but was not found in the endosperm of rice grain when investigated using LA-ICP-MS.

Several studies have previously determined the *species of Sb present in Glucantime*. However, low resolution between N-methylglucamine (NMG-Sb^{V}) and iSb^{V} has often been problematic. Additionally, the stability of iSb^{III} during sample treatment may also cause problems. The development of an analytical methodology for the separation of the Sb species of NMG-Sb^{V}, iSb^{V}, and iSb^{III} in Glucantime which overcomes these issues has been reported by Roldan *et al.*[36]. The revised method used anion exchange HPLC-HG-AFS in gradient elution mode with different concentrations of Na_2H_2EDTA as the mobile phase. The best results were obtained with a 1.20 min elution of 0.473 mmol L^{-1} Na_2H_2EDTA as the first mobile phase for the separation of NMG-Sb^{V}, which was then changed to 20 mmol L^{-1} for the elution of the iSb species. The results revealed that the iSb^{III} content may be underestimated when sample filtration is used prior to the separation step. Instead of filtration, this study demonstrated that an adjustment to pH 9 and dilution with deionized water are a better strategy to determine the iSb^{III} content. The method proved to be reproducible for urine samples from rats injected intraperitoneally with NMG-Sb^{V}.

3.2 Arsenic

Although As speciation remains a popular area of study, the overall number of publications reporting on As speciation has declined significantly this year. Most reports focusing on *method development for As speciation* during the review period have presented modifications to existing methodology. One exception is the use of frontal chromatography (FC) coupled to ICP-MS for the first time[37]. In FC, the concentration of an analyte is estimated from frontal sigmoidal curves which can be seen as the integral of a conventional elution chromatogram. The principal benefits of the FC-ICP-MS approach are reported to be the short analysis time and the simple instrumental setup. In this study two different chromatographic systems were evaluated with each differing in the way the sample was fed to the ICP-MS instrument. The first arrangement had a direct connection from a strong anion exchanger at pH 7.5, used to separate As^{III} and As^{V}, to the nebuliser. However, the loading capacity of the nebuliser did not allow shortening of the retention times and so a T-shaped connector was inserted after the column to divert some of the sample to waste. A peristaltic pump delivered the sample to the column and the solution exiting the column was mixed with 4% HNO_3 before reaching the nebuliser. After the optimisation of the instrumental setup and the frontal chromatographic parameters, As^{III} and As^{V} concentrations up to 240 µg kg^{-1} could be determined within 120-140s using either univariate or multivariate calibration approaches. Quantification using the slopes of the inflection points provided good accuracy and linearity for both species, but the utilisation of a multivariate approach was better still. The best results were obtained using PLS calibration and gave LOD values of 0.18 and 0.21 µg kg^{-1} for As^{III} and As^{V}, respectively.

As reported last year, the number of reports on *non-chromatographic methods for As speciation* is increasing, although the idea of using selective reduction for As speciation is certainly not new or novel. A method to determine iAs in rice using species-selective HG-ICP-OES without prior separation of methylated As species has been developed and validated[38]. Four As species, As^{III}, As^{V}, DMA and MMA, were extracted in aqua regia to oxidize As^{III} to As^{V}, whilst preserving the integrity of both methylated forms. Hydrides from iAs were generated by reaction with $NaBH_4$ (1%) in strong acidic conditions (10 mol L^{-1} HCl) after pre-reduction of As^{V} to As^{III} in a KI (0.5%)-ascorbic acid (2%)-HCl (3 mol L^{-1}) mixture. The reactivity of As species for HG within a rice matrix was investigated in order to improve detectability and selectivity of iAs when methylated As coexist. A LOD of 0.28 ng $g^{-1}$was obtained for iAs (5.6 ng g^{-1} in original sample), with a precision <5%. The accuracy as verified by an analyte recovery study was 97-103%. The use of HG-AFS has also been optimized for the determination of As^{III}, As^{V}, DMA^{V} and MMA^{V} in rice, again using non-chromatographic speciation [39]. For optimum results, HCl/sodium citrate buffer (1.6 mol L^{-1} sodium citrate; pH 4.8) was used for the carrier solution and 0.06 mol L^{-1} citric acid for sample acidification. The concentrations of the four As species were determined by HG-AFS using a series of linear independent equations corresponding to four different sample pre-treatment procedures. The LOD values of the method were 0.21, 0.52, 0.65 and 0.9 µg kg^{-1} for As^{III}, As^{V}, MMA^{V} and DMA^{V}, respectively. Extraction efficiencies (96-104%) and recoveries of As species in rice (95-100%) were good, and interconversion among the four As species in the extract did not occur. An on-line non-chromatographic HG-AFS system has been described for the determination of As^{III} and As^{V} species in drinking water[40]. Thioglycolic acid was used for on-line pre-reduction of As^{V} and quantification of As species was accomplished via calibration with As^{III}. Samples and calibration standards were prepared in Tris buffer (pH:7.2) for selective HG without on-line pre-reduction. The As^{V} signal was found to be negligible up to 150 ng ml^{-1}. The LOD values for As^{III} and As^{V} were 27 pg ml^{-1} and 36 pg ml^{-1} respectively. Recovery studies were performed and the CRM

TMDA-61 (trace elements in lake water) was used to test the accuracy of the method. The main form of As in all water samples was As^{V} and As^{III} concentrations were below the LOD for some samples. A multi-walled carbon nanotubes-branched PEI adsorbent composite material has been employed to separate and pre-concentrate As^{V} in water samples[41]. The characterization of the material by XRD, TGA, FTIR and Raman spectroscopy showed successful modification of the composite which exhibited selective retention of As^{V} in the presence of As^{III} in water samples of pH 7 using 40 mg of composite as adsorbent. The pre-concentrated As^{V} was quantified using ICP-MS. A LOD of 0.0537 μg L^{-1} and LOQ of 0.179 μg L^{-1} were achieved along with a pre-concentration factor of 23.3 and a percentage recovery of 81.0%. The As^{V} concentrations in water samples were in the range of 0.0612 - 3.65 μg L^{-1}. The As^{V} concentrations determined using an alternative SPE procedure were in good agreement with the concentrations obtained using HPLC-ICP-MS. A LLE technique using an aqueous two-phase system formed by a polymer and electrolyte for the extraction, determination, and speciation analysis of iAs has been reported[42]. The pH, nature of the aqueous two-phase system forming the electrolyte and the polymer, tie-line length (TLL) of the system, type and concentration of extractants, and the mass ratio of the top and bottom phases were optimised. The highest extraction (98%) was obtained for an aqueous two-phase systems composed of copolymer L64 (poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) with sodium sulphate and water at pH 6.0, tie-line length 33.55 % w/w using APDC extractant in a molar ratio (APDC/As^{III}) of 960, and with a mass ratio of 1/4. Using these same conditions, As^{V} was not well extracted (18%) after three extractions. The method was validated using HG-ICP-OES and gave a LOD of 0.20 μg kg^{-1}. When applied to spiked tap water samples, recovery was 87.10%. An eutectic solvent based vortex assisted microextraction procedure for the separation and preconcentration of low levels of As from sample matrix (waters, honey and rice) prior to analysis by HG-AAS has been reported[43]. The type, composition and volume of the deep eutectic solvents was first optimised and the best recoveries obtained with a molar ratio of 1:3 (choline chloride: oxalic acid), and a volume of 700 µL. The effect of pH, choice of aprotic solvent, vortex time and sample volume were also optimised. The As^{V} was reduced to As^{III} prior to determination of the total iAs. The LOD, RSD and recoveries and enhancement factor for the method were calculated as 7.5 ng L^{-1}, 2.1%, 93.5% and 104, respectively. Method validation was successfully carried out with the CRM 1568a rice flour and SRM 1643e trace elements in water.

The determination of *Arsenic in food* has continued to attract attention over this review period, although in a departure from the trend in recent years, As in rice and rice products have received a lot less attention this year. Rice as a matrix for method development studies continues to be popular as noted above. A market basket survey of As species in the top ten most consumed seafoods in the United States has been reported[44]. Fifty-four samples were collected from local supermarkets, and their species identities were confirmed by DNA barcoding. The total As in the samples varied greatly in the range of 8-22200 ng g^{-1} (wet mass). Speciation analysis based on extraction of water-soluble and nonpolar As showed that iAs was found only in clams and crabs, while AB predominated in most samples. Among the other arsenicals, trimethylarsoniopropionate (TMAP) was found in most matrices with higher concentrations in crabs, and arsenosugars existed in most clams and crabs. Nonpolar As accounted for 1-46% of the total As in the samples. The accuracy of the analytical results was evaluated using SRMs and spike recovery tests. The survey showed that the iAs concentrations in America's most consumed seafood products are much lower than the tolerable intake set by the Joint FAO/WHO Expert Committee. Matrix-induced transformation of As species in seafoods have also been studied by the same group[45]. Sixteen arsenicals were individually spiked into samples of finfish, crustaceans and molluscs. The spiked samples were subjected to a hot water extraction at 90 °C, and extracts were then analysed by HPLC-ICP-MS. Seven As species, As^{V}, AB, AC, DMA, MMA, TMA and TMAP, remained intact in all the matrices. However, As^{III}, DMA, dimethylarsinoyl ethanol (DMAE), dimethylarsinoyl propionate (DMAP), TMAO and glycerol-, sulfonate-, sulfate- and phosphate-arsinoylribosides (arsenosugars 328, 392, 408 and 482, respectively) were transformed to other species in most finfish and crustaceans. The transformation of the arsenicals was found to be induced by matrix thiols. While As^{III} was bound to sulfhydryl groups, DMA, DMAE, DMAP, TMAO and arsenosugars 392, 408 and 482 were thiolated through conversion of their arsinoyl (As=O) functionalities to arsinothioyl (As=S). The newly formed arsinothioyl compounds were characterised by HPLC-ICP-MS and HPLC-ES-HR-MS-MS. The observed matrix-induced transformation of the As species could be prevented by treating the samples (prior to spiking) with the thiol-selective blocking agent, N-ethylmaleimide. In a third publication from the US Food and Drug Administration's team, various issues encountered in the determination of As in fruit juice are discussed and a solution using a modified extraction procedure reported[46]. Juice samples were subjected to total As determination and those containing As > 10 µg kg^{-1} were subjected to As speciation analysis using FDA Elemental Analysis Manual (EAM) 4.10 method (AOAC First Action Method 2016.04), basically a MAE followed by HPLC-ICP-MS analysis, to determine the concentration of iAs and other common organic As species. For a subset of samples, the sum of the As species was significantly less than the total As value (*i.e.*, mass balance < 65%), uncommon for a liquid-based matrix. The group report that juice types that have previously exhibited this behaviour include pomegranate, prune, and cherry juices. Causes for this issue were explored which ultimately led to an alternate sample preparation technique using an extraction with 0.28 mol L^{-1} HNO_3 along with heat, which resulted in drastically improved mass balance approaching 100%. The method proved robust, with both accurate and precise measurements for multiple juice samples reported by four laboratories during a multi-laboratory validation exercise.

Further reports on *As speciation in foods* include the determination of Total As, As^{III}, As^{V}, DMA and MMA have been determined in hypoallergenic formula infant food and grain porridges commercially available from a Polish market[47]. After quantitative extraction with 0.5% HNO_3, separation of individual species was performed by HPLC, and then determined by NAA and/or ICP-MS. Due to relatively low concentrations of As in the analysed samples, it was only possible to determine DMA using HPLC-ICP-MS. An HPLC separation coupled with off-line determination by NAA enabled the determination of more extracted As species (especially iAs) with good accuracy. The products that contained rice had the highest levels of iAs. The SRM, NIST 1568b Rice Flour, was used for the validation of both procedures. The mobility of iAs and its species in selected herbs has been studied[48]. The aim of the study was to investigate the response of chamomile (*Matricaria recutita (L.) Rauschert*), peppermint (*Mentha x piperita*), lemon balm (*Melissa officinalis L.*), and sage (*Salvia officinalis L.)* to elevated iAs in soils. The ability of herbs to accumulate As was tested in pot experiments in which the soils were spiked with As^{III} and As^{V}. The As^{III}, As^{V}, AB, MMA and DMA were determined by HPLC-ICP-MS with separation on a Hamilton PRP-X100 column. The study determined the total As contents in the soil and plants, as well as the mobility of the As species from the soil into the studied plants. Peppermint demonstrated the highest As concentrations and phytoaccumulation amongst the plants studied. Sequential chemical extraction showed that As in the contaminated soil was mainly related to the oxide and organic-sulphide fractions, and that the oxidized As form had a greater ability to accumulate in herbs and was more readily absorbed from the substrate by plants. Soils contaminated with As^{III} or As^{V} had different effects on the iAs content in plants, with most samples also having elevated levels of MMA and AB, particularly in the stems. In a further study, As^{III} and As^{V} in phosphate-rich *in vitro* bio accessible fractions of Ayurvedic formulations (Hindu medicines containing both plant and mineral extracts) have been investigated[49]. Microwave-assisted acid digestion and ICP-MS with off-line ion exchange SPE procedures were used to discriminate between iAs species. A range of elements (including As, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Sn and Zn) were determined in the µg kg^{-1} to mg kg^{-1} range, with As between 0.081- 106 mg kg^{-1}. The bioaccessible As fraction from the mineral formulations reached about 10% by weight, and was assessed using pepsin and pancreatin model suspensions of pH1.2 and 6.8 (with 0.05 mol L^{-1} K_2HPO_4), respectively. The bioaccessible As fraction of the mineral formulations was about 10-20%. Thus, As intake from these products greatly exceeded the 3.0 µg kg^{-1} BW day^{-1} benchmark dose limit recommended by the WHO. An overview of As in wine has been presented by Tanabe *et al.*[50]. This review style paper with 36 references considered As in soil and uptake into vines, typical concentrations of As species found in wine, the influence of processing on total As levels and speciation, analytical approaches for determining As species in wine, and some thoughts on future research needs. International regulatory bodies have imposed allowable maximums for total As in wine ranging between 100 and 200 µg L^{-1} with typical commercial wine levels falling within these limits. The speciation of As, Sb, and Cr in flavoured bottled drinking water samples using HPLC-ICP-MS and ES-MS-MS has been reported[33]. The study builds on previous work by the same authors, but focuses on a different sample type. Five As species, AB, As^{III}, As^{V}, DMA and MMA were analysed for and found to be in the range from 0.286 ± 0.027 g L^{-1} to 0.414 ± 0.039 g L^{-1} for As^{III}, and from 0.900 ± 0.083 g L^{-1} to 3.26 ± 0.30 g L^{-1} for As^{V}. No DMA or MMA was detected in any sample and AB ranged from 0.0541 ± 0.0053 g L^{-1} to 0.554 ± 0.054 g L^{-1}. For non-targeted speciation, SEC-ICP-MS was used as a complementary technique, with ES-MS-MS. No unknown species were detected.

Arsenic speciation in mushrooms has been studied for more than 20 years. A critical review of this area with a focus on analytical methods has been published by Braeuer and Goessler[51]. The As species found in mushrooms to date are summarised, and the origin of the different species discussed in some depth. The most common analytical technique used is reported to be HPLC-ICP-MS, but a few alternative approaches exist and these are also discussed. Additionally, the phenomenon of As hyperaccumulation by mushrooms is also reviewed. In another publication[52], the occurrence, toxicity, and speciation analysis of As in edible mushrooms has also been reviewed. Covering just over 100 articles, the review highlights recent advances in As speciation analysis with respect to edible mushrooms and discusses both the concentration and distribution of As species within mushrooms. Methods for sample preparation, and techniques for identification and quantification were covered. The stability of As species during sample pre-treatment and storage is also discussed. Arsenic speciation in three species of mushrooms (*Boletus edulis*, *Tricholoma matsutake* and *Suillellus luridus*) collected in Yunnan, China, has been reported[53]. The purpose of the study, which employed both IC and SEC coupled to an ICP-MS detector, was to determine if the As in the mushrooms posed a threat to human health. Microwave assisted extraction, UAE and enzymatic assisted extraction were evaluated, and applied in a three step study looking at a simulation of mouth digestion; a simulation of mouth and gastric digestion; and a simulation of mouth, gastric and intestinal digestion. Microwave assisted extraction gave slightly better extraction efficiencies (from 75% to 90%) than for UAE. The enzymatic assisted extraction was used to estimate the bioaccessibility *via* application of a modified BARGE bioaccessibility method for *in vitro* studies and SEC-UV-VIS/ICP-MS analysis was used for these extracts. The main signal was obtained at a molecular mass of about 5 kDa for all mushroom extracts. Monitoring of an $^{50}SO^+$ ion confirmed that this signal came from As-proteins. Five as species AB, As^{III}, As^{V}, DMA and MMA were found in all samples, with an additional unknown As species also identified in the *Boletus edulis* sample. A study of the transformations of AB, AC, As^{III}, As^{V}, DMA and MMA in edible mushrooms following ultrasonic extraction in aqueous solution has been reported[54]. The transformations of As species increased with time. As the ultrasonic power or temperature was increased, transformations of As species was at first enhanced and then decreased, with AC, AB, As^{III} being the most unstable at 600 W and 20 °C. It was found that DMA and MMA were stable whilst AC was partially transformed to AB and TMAO, and TMA^+ and AB were partially transformed to TMAO. The As^{III} present was easily oxidised to As^{V}. The reduction of As^{V} to As^{III} was only observed when the temperature was over 60 °C. The study may serve as a guide for improving extraction efficiency with minimal transformation of As in edible mushrooms. The concentrations, speciation, and localisation of As in cultivated market mushrooms from markets in nine cities in China, have been reported[55]. A total of 141 samples of *Lentinula edodes*, *Pleurotus ostreatus*, and *Agaricus bisporus* were used in the study which focused on human health implications. The US EPA 3050B method, employing NHO_3 and 30% H_2O_2, was used for sample preparation followed by HPLC-ICP-MS to determine AB, As^{III}, As^{V}, DMA and MMA. Total As amounts ranged from 0.01 to 8.31 mg kg^{-1} dw, with *A. bisporus* (0.27-2.79 mg kg^{-1}) containing the most As followed by *P. ostreatus* and *L. edodes* (0.04-8.31 and 0.12-2.58 mg kg^{-1} respectively). However, As in *A. bisporus* was mostly found as organic As species, while *P. ostreatus* and *L. edodes* contained mainly iAs. Following *in situ* imaging using LA-ICP-MS, As in *L. edodes* was found to be localised in the surface coat of the cap, while As in *P. ostreatus* was localised to the junction of the pileus and stipe. When As speciation and daily mushroom consumption (1.37 g d^{-1} dw) are considered, daily mushroom consumption of these samples may result in elevated iAs exposure with associated health risks.

Arsenic speciation in seafood continues to attract attention. A study of iAs and total As in rice, fish and shellfish products from a Swedish market has been undertaken[56]. For the speciation of iAs the European standard EN:16802 based on AEC-ICP-MS was used. The two market basket food groups, cereals (including rice), and sweets and condiments (a mixed group of sugar, sweets, tomato ketchup and dressings), contained the highest iAs levels (means 9 and 7 µg iAs kg^{-1} respectively), whereas other food groups, including fish, did not exceed 2 µg iAs kg^{-1}. Varying levels of iAs were found in separate samples of tomato ketchup, 2.4-26 µg kg^{-1}, and it was suggested to be one reason for the rather high average level of iAs in the food group sweets and condiments. Some specific food products revealed much higher iAs levels, *i.e*. Norway lobster and rice crackers were 89 and 152 µg iAs kg^{-1} respectively. In the same study, the intake of iAs via food was estimated using data from two national consumption surveys, performed in 2010-11 (1797 adults) and 2003 (2259 children). The estimated median iAs intake for adults and children were 0.047 and 0.095 µg kg^{-1} body weight day^{-1}, respectively. The iAs intake for rice eaters was about 1.4 times higher than for non-rice eaters. Validation of the consumption survey-based iAs intake, using food purchase and market basket data mainly from 2015, resulted in a per capita intake of 0.056 µg kg^{-1} body weight day^{-1}. The estimated cancer risk for adults using low-dose linear extrapolation was <1 per 100,000 $year^{-1}$. A validation exercise and inter-laboratory study of selective HG-ICP-MS after microwave-assisted extraction with diluted HNO_3 and H_2O_2 for fast screening of iAs in seafood has been reported[57]. High concentrations of HCl (8 mol L^{-1}) for HG along with H_2O_2 in samples of a similar concentration as used for extraction led to a selective conversion of iAs to volatile arsine. A contribution from MMA^V (approximately 20% of the iAs signal) was found, while HG from DMA^V and TMAO was substantially suppressed (less than 1% relative to iAs). The methodology was applied to TORT-3, DORM-3, DORM-4, DOLT-4, DOLT-5, PRON-1, SQID-1 and ERM-CE278k CRMS and resulted in iAs being determined for the first time in some of the samples. Various seaweed samples from a local store were also analysed. The results were compared with a reference method and the selectivity of the iAs determination was evaluated. The inter-laboratory reproducibility was tested by comparative analyses of six fish and four seaweed samples in three European laboratories, with good agreement between the results. The method (LOD 2 mg kg^{-1} iAs) proved suitable for screening a large number of samples and selective for determining iAs concentration levels, useful if maximum limits are set into EU legislation for marine samples. Variation in the arsenolipid concentration in seafood has been assessed in 18 seafood samples including fish, shellfish, and crustaceans in a study on seafood consumed in Japan[58]. Analyses were performed by HPLC-ICP-MS/ES-MS-MS. Stable isotope ratios for N and C were also measured in the samples to obtain trophic level information of the species. Arsenohydrocarbons (AsHCs) and As-containing fatty acids (AsFAs) were detected in the seafood samples; AsHCs were found in all of the seafood samples with a large variation in the concentrations (83 ± 73 ng As g^{-1}, coefficient of variation = 88%). The AsHC concentrations correlated with the lipid content of the seafood samples (r = 0.67,

$p < 0.01$), reflecting the fat solubility of the compounds. The AsHCs concentrations did not correlate with N stable isotope ratios suggesting that AsHCs do not biomagnify. The source of the large variation in AsHC concentrations is the subject of further investigation. Method development and an health assessment of As speciation in edible shrimp has been reported[59]. Analysis of MAE sample extracts by HPLC-ICP-MS used a collision/reaction cell in the O_2 mass-shift mode to remove interferences. Six As species were detected within 11 min after optimisation with LOD values of 0.010 to 0.028 µg L^{-1} and precisions in the range 1.89 to 3.14%. The results showed that AB was the predominant As species and the iAs content was below 0.5 mg kg^{-1} in the shrimp samples analysed, thus presenting no health risk to local consumers. The simultaneous determination of iAs and MeHg in shellfish using MAE and HPLC-ICP-MS quantification has been reported for sites near coastal cities (Lianyungang and Yantai) in China which potentially have high levels of pollution[60]. The concentrations of iAs and MeHg in fresh shellfish samples were 0.10-1.69 mg kg^{-1} and 0.46-6.38 µg kg^{-1}, respectively. The target hazard quotient and target cancer risk values of iAs were the highest for the children and adults in Lianyungang, and were larger than 1 and 1×10^{-4} respectively, indicating that both non-carcinogenic and carcinogenic risks of iAs exposure were significant in this area.

Seaweed is a common matrix for studies on As speciation, possibly because of the growing demand for edible seaweed and a knowledge that brown seaweeds, such as *Laminaria digitate*, are known to accumulate As to concentrations of more than 100 mg kg^{-1}. A review on As in edible seaweed covering articles published up to 2019 has been published by Camurati and Salomone[61]. Topics included the extraction methods and analysis techniques most frequently used for seaweed and the need to develop CRMs for validation purposes. The most common As species found in marine macroalgae are also discussed along with the potential effects on human health and the current legislation in relation to the risk associated with consumption. Despite this interest in seaweeds and the growth in the number of publications, there are still gaps in our understanding of As biochemistry. Ender *et al*.[62] have reported on the novel use of high resolution imaging using nanoSIMS and TEM to elucidate the molecular structures of As species and the location of the As in cells and cellular substructure in *Laminaria digitate.* The majority of the 117 mg kg^{-1} As found in *L. digitata* fronds was in the form of iAs (53%) and arsenosugars (32%) and only 1.5% of the total As as arsenolipids (mainly as AsHC and arsenosugarphospholipids AsPL). Using nanoSIMS with a lateral resolution of 300 nm, the majority of As was found in the cell walls and cell membrane, while the inside of the cell was almost As free. However, the nanoSIMS images raised questions regarding the integrity of the As species during extraction and indicate that iAs is unlikely to occur freely in the seaweed. Whether iAs and the arsenosugars were bound directly to the polymeric carbohydrate alginates or fucoidans in the seaweed was unclear and needs further investigation. The same group[63] have also published on the distribution of arsenolipids within two brown macroalgal species *Saccharina latissima* (30-40 individuals) and *Alaria esculenta* (15-20 individuals) collected from natural populations in winter in Iceland. The algal thalli were sectioned into different parts (e.g. holdfast, stipe, old frond, young frond and sori-containing frond sections) that differed in age and biological function. The study showed that As was not uniformly distributed within the two brown macroalgal species, with lower levels of total As found in the stipe/midrib compared to other thallus parts. The arsenosugars mirrored the total As in the seaweed mainly due to $AsSugSO_3$ being the most abundant As species. However, As speciation using parallel HPLC-ICP-MS/ES-MS showed that the As-containing lipids had a different distribution where the As-containing lipids differed by approximately a factor of 4 between the sections containing the lowest and highest concentrations. When placing the sections in order of metabolic activity and estimated tissue age, there appeared to be a relationship between the activity and As-containing lipids, with lower levels of As-containing lipids in the oldest parts. This is the first time such a relationship has been shown for As-containing lipids. A method validation exercise has been reported by Matsumoto-Tanibuchi *et al*.[64]. Samples of seaweed and seafood were heated at 100 °C in 0.3 mol L^{-1} nitric acid to extract As species which were quantified by LC-ICP-MS using an ODS column with a mobile phase containing an ion-pair reagent. The LOD values of 0.0023-0.012 mg kg^{-1} were obtained with a LOQ of 0.0077-0.042 mg kg^{-1}, repeatability of 3.0-7.4%, precision 4.4-7.4%, and recoveries of 94-107%. Inorganic As was detected in almost all of the evaluated dried seaweed products, including the Japanese oyster, nam pla, oyster sauce, and the intestinal organs of seafood. The brown algae akamoku (*Sargassum horner*), hijiki (*Sargassum fusiforme*), and mozuku (*Cladosiphon okamuranus*) had the highest As content. A revised method to quantify AC, AB, As^{III}, As^{V}, DMA, and MMA in seaweed, sediment, and seawater samples using HPLC-ICP-MS with a collision/reaction cell to eliminate spectral interferences has been reported[65]. Ammonium nitrate- and phosphate-based eluents were used as mobile phases offering an overall runtime of 6 min with good peak separation. The As species were extracted with a 1% HNO_3 solution, requiring no clean-up process. The optimised method was verified by applying it to a hijiki seaweed CRM, NMIJ 7405-a, and to spiked blank samples of sediment and seawater. The proposed method was used to determine the concentration of As^{V} in the CRM, 9.6 ± 0.6 µg kg^{-1}, which is close to the certified concentration of 10.1 ± 0.5 µg kg^{-1}. The recovery of the six spiked As species was 87-113% for the sediment and 99-101% for the seawater. In the analysis of real samples, As^{V} was the most abundant As species present in hijiki and gulfweed, whereas AB was dominant in other seaweed species. The most dominant As species in the sediment and seawater samples were As^{III} and As^{V}, respectively.

Studies focusing on *As speciation* in *environmental samples* have been diverse in nature over this review period. Freshwater aquatic environments close to cities and industrial areas tend to be at higher risk of environmental pollution. The freshwater shellfish *Semisulcospira cancellate* has been introduced as a bioindicator to monitor the heavy metal contamination at four city cites on the mine-impacted Xiang River in China[66]. The concentrations of a range of metals were determined by ICP-MS and multivariate statistical analyses such as Pearson's correlation analysis and PCA were employed to identify the possible sources of the elements in the shellfish samples. The shellfish samples were then categorized according to the sampling sites with different contamination levels. Six As species, AB, AC, As^{III}, As^{V}, DMA and MMA, were separated and quantified using HPLC-ICP-MS. The concentrations of As^{III} and As^{V} increased linearly with total As concentration. However, the proportion of AB decreased with total As. Total As, As^{III}, As^{V}, DMA, MMA have also been determined in freshwater fish samples collected from the Wielkopolska and Lower Silesia provinces in Poland[67]. Both ICP-MS and HPLC- ICP-MS were used, with oxygen as a reaction gas, for these analyses. Sample preparation, digestion, and extraction using UAE and MAE were optimised and the method was validated using four CRMs (BCR627 Tuna fish tissue, MODAS-3 Herring tissue, MODAS-5 Cod tissue and ERM-BB422 Fish mussel). The As species were separated in 8 min on an AEC Hamilton PRP-X100 column using 10 mmol L^{-1} ammonium dihydrogen phosphate and 10 mmol L^{-1} of ammonium nitrate as the mobile phase. The elution order was AB, As^{III}, DMA, MMA, As^{V}, and to overcome some overlap of AB and As^{III} in the presence of a high concentration of AB and trace amounts of As^{III}, the conditions were modified by adding 20 mmol L^{-1} of ammonium nitrate at pH 8.6 to facilitate full separation within 4 minutes. The LOD values were in the range 0.056 µg L^{-1} for total As to 0.15 µg L^{-1} for As^{V}. The reactivity of co-occurring As and U in mine wastes in NE Arizona has been investigated using batch reactors, microscopy, spectroscopy, and aqueous chemistry[68]. Analysis with XRF and electron microprobe showed the co-occurrence of As and U with Fe and V. Field measurements found As (< 0.500 - 7.77 µg L^{-1}) and U (0.950 -165 µg L^{-1}) in waters, as well as mine wastes (< 20.0 - 40.0 mg kg^{-1} As and < 60.0 - 110 mg kg^{-1} U) and background solids (< 20.0 mg kg^{-1} As and < 60.0 mg kg^{-1} U). Later laboratory experiments indicated that labile and exchangeable As^{V} was released into solution when solids were sequentially reacted with water and $MgCl_2$, while limited U was released to solution with the same reactants. The predominance of As^{V} in mine waste solids was confirmed by XANES analysis. Both As and U were released to solution after reaction of solids in batch experiments with HCO^{3-}. Both XPS and XANES analysis determined the predominance of Fe^{III} in the solids. Mossbauer spectroscopy detected the presence of nano-crystalline goethite, Fe^{II} and Fe^{III} in (phyllo)silicates, and an unidentified mineral with parameters consistent with arsenopyrite or jarosite in the mine waste solids. The results suggested that As and U could be released under environmentally relevant conditions in mine waste, and thus applicable to risk and exposure assessment. Arsenic speciation in coal samples from the Velenje coal mine in Slovenia, one of the largest actively mined coal basins in Central Europe has been studied by Kanduc *et al*.[69]. The samples were divided into organic rich and inorganic rich fractions according to the percentage of carbon. Speciation analysis revealed the presence of several organoarsenic compounds in the organic rich samples, with TMA (0.01-1.10 µg g^{-1}) and TMAO (0.01-0.29 µg g^{-1}) as the most abundant. A comparison with coal samples from the Senovo, Kanaarica, and Trbovlje coal mines in Slovenia and from the Sokolov Basin (Czech Republic), revealed that Velenje coal contains a much higher amount of organoarsenic compounds (34.8 ± 16.9%) in comparison to the others (4.45 ± 4.19%). The removal of As^{V} from water using an Al-based adsorbent and coal mine drainage sludge coated polyurethane prepared using alum and coal mine sludge, respectively has been described[70]. The As^{V} removal efficiencies obtained were compared with each other and with granular ferric hydroxide. The mineralogy and surface chemistry of the materials were determined using WD XRF and FTIR, respectively. Angle-resolved XPS was used to confirm As^{V} retention on the adsorbent surfaces. The adsorption kinetics data were fitted to pseudo second-order rate equation. The faster As^{V} uptake kinetics of granular ferric hydroxide and Al-based adsorbent (granular ferric hydroxide > Al-based adsorbent > coal mine drainage sludge coated polyurethane) were attributed to their large pore volume and mesoporous nature. Langmuir adsorption capacities of 22, 31 and 10 mg g^{-1}, were achieved for granular ferric hydroxide, Al-based adsorbent and coal mine drainage sludge coated polyurethane, respectively. As^{V} adsorption on granular ferric hydroxide, Al-based adsorbent and coal mine drainage sludge coated polyurethane was endothermic. Granular ferric hydroxide and Al-based adsorbent were efficient over the pH range 3-10. When using column studies, granular ferric hydroxide, Al-based adsorbent, and coal mine drainage sludge coated polyurethane successfully treated As^{V} contaminated water (pH = 6.0, iAs = 600 µg $L^{-1)}$. The authors suggested that both the granular ferric hydroxide and the Al-based adsorbent have potential to be used for large-scale aqueous phase As^{V} remediation.

Further *reports on As speciation in the environment* include those species found in wood used in playground structures. Those built prior to 2004 were often been pressure-treated with chromated copper arsenate (CCA) which has been associated with negative health and environmental impacts.

Given the prevalence and lack of maintenance of these ageing play structures in rural NE USA, a study has been conducted to determine the distribution of As (total, speciated and bioaccessible) in surface soil collected near and underneath four CCA-treated playground structures, 16 and 26 years after installation[71]. Total As in surface soil was measured by ETA-AAS, whereas As speciation and bioaccessible As were determined by HPLC-ICP-MS and an *in vitro* solubility / bioavailability research consortium (SBRC)-gastric assay, respectively. Near (≤5 cm) and underneath CCA-treated structures, the total As concentration in surface soil ranged from 143 - 214 mg kg^{-1} after 26 years of installation compared to 101-167 mg kg^{-1} ten years later. These concentrations exceed the Massachusetts Residential Risk-Based Soil Standard by 5-10 times. In comparison, total As in background soil samples ranged from 4.6 - 6.6 mg kg^{-1} during the two study periods. While most of the As in the surface soil was in the form of As^{V}, ≤ 29% was bioaccessible. Overall, the findings demonstrated that As accumulation in soil surrounding aging playground structures continues to be a source of elevated exposure to children through contact with contaminated soil. A study to assess the health risk associated with two common As exposure routes, drinking water and soil ingestion, in children living in the most important agricultural areas in the Yaqui and Mayo valleys in Sonora, Mexico, has been reported[72]. Drinking water samples were collected from the wells of 57 towns, and the cross-sectional study included 306 children from 13 villages in the valleys. The water samples and first morning void urine samples were analysed for iAs, DMA and MMA by HPLC-ICP-MS. The results showed a wide range of As levels in drinking water, ranging between 2.7 and 98.7 mg As L^{-1}. The As levels in agricultural and backyard soils were in the range of <10 – 27 mg As kg^{-1}. The hazard index (HI) and hazard quotient (HQ) for drinking water, agricultural soil, and backyard soil showed values >1 for all of the towns studied. The average As excreted in urine was 31.7 mg L^{-1}, with 77.8% of this DMA, and 11.4 and 10.9% as iAs and MMA, respectively. The results clearly indicated that this population is at high risk of developing chronic diseases including cancer.

As speciation in urine is critical to reveal the metabolic mechanism and the relationship between As species and the clinical response when As_2O_3 is used for the treatment of acute promyelocytic leukaemia. To characterize the As species (As^{III}, As^{V}, DMA and MMA) in urine from such patients, a robust HPLC-HG-AFS method has been developed, validated and used for urine samples from 66 patients with acute promyelocytic leukaemia[73]. Patients received As_2O_3 (0.16 mg kg^{-1} day^{-1}) via continuous slow-rate infusion or conventional infusion. Urine samples were collected at steady state before the start of the next daily administration. The relative proportions (median) of As species in urine were: As^{III}, 33% (IQR: 24-46%); DMA^{V}, 36% (IQR: 25-52%); MMA^{V}, 23.89% (IQR: 20-27%); and

As^{V}, 2.22% (IQR: 1.3-3.7%). The levels and proportions of As species varied widely among individual patients. Good positive correlations were found between the levels and proportions of As species in urine and those found in plasma; thus, urinary As could be used to reflect the levels of As in plasma. The same group have also reported on a study of the effect of continuous venovenous haemodialysis on outcome and pharmacokinetics of As species in a patient with acute promyelocytic leukaemia and acute kidney injury[74]. Species of As_2O_3 metabolites in plasma and effluent were analysed using the method above. Plasma concentrations of As^{III}, DMA and MMA after haemodialysis were lower than those found before haemodialysis. The area under the concentration-time curve from 0 to the last sample with quantifiable concentration of As^{III} without continuous venovenous haemodialysis was significantly higher than that with continuous venovenous haemodialysis (292 ng h mL^{-1} vs 196 ng h mL^{-1}, P = 0.037), which was not observed for MMA and DMA. Dialysate saturation of As species was remarkable, especially for As^{III} and complete remission was achieved and renal function recovered. The study indicated that As_2O_3 could be used safely and effectively to treat acute promyelocytic leukaemia patients undergoing continuous venovenous haemodialysis without dose adjustment. Urinary As profiles and DNA hypomethylation in a hospital-based case-control study of urothelial carcinoma have been reported by Chung *et al*.[75]. The aim of the study was first to explore the effect of interactions of urinary total As levels, As methylation capacity, 8-hydroxy-2 '-deoxyguanosine (8-OHdG), plasma folate, and global 5-methyl-2 '-deoxycytidine (5-MedC) levels on the risk of urothelial carcinoma. The research involved the histological recruitment and pathological verification of 178 urothelial carcinoma patients and 356 age / sex-matched controls without prior history of cancer. Arsenic species were determined by HPLC-HG-AAS. The 5-MedC levels were detected by HPLC and triple-quadrupole MS, and 8-OHdG by online SPE LC-MS-MS. Plasma folate levels were measured using chemiluminescent technology. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by multiple logistic regression analysis. The results indicated that the high levels of total urinary As, iAs percentage, and 8-OHdG and the low levels of DMA % and plasma folate were independent factors of urothelial carcinoma. In addition, global 5-MedC levels in the first quartile versus fifth quartile significantly increased the twofold OR of urothelial carcinoma after potential factors were adjusted (95% CI:1.10-4.03). The interaction of 5-MedC level and high total As level, As capacity, high 8-OHdG, and low folate levels were insignificant. The results of stepwise logistic regression analysis indicated that high total urinary As levels (Q3 versus Q1), low plasma folate level, and low global 5-MedC (Q4 versus Q5) significantly increased the ORs of urothelial carcinoma. The results suggested that high total As, low plasma folate, and 5-MedC levels affect the ORs of urothelial carcinoma independently. A study to assess human exposure to As in well water in villages around Lake Poopo in Bolivia has been reported with the aim of elucidating whether the metabolism and detoxification of As in this population was as efficient as that previously indicated for other Andean areas[76]. The study recruited 201 women from 10 villages around Lake Poopo. Arsenic exposure was determined as the sum concentration of As metabolites (iAs, DMA and MMA) in urine measured by HPLC-HG-ICP-MS. The efficiency of As metabolism was assessed by the relative fractions of the urinary metabolites. The women had a wide variation in urinary As (range 12 - 407, median 65 µg L^{-1}) and a markedly efficient metabolism of As with low % MMA (median 7.7%, range: 2.2 -18%) and high % DMA (80%, range: 54 - 91%) in urine. In multivariable-adjusted linear regression models, ethnicity (Aymara-Quechua *vs.* Uru), body weight, fish consumption and tobacco smoking were associated with urinary As metabolite fractions. On average, the Uru women had 2.5% lower iAs, 22% lower MMA and 4.7% higher DMA compared with the Aymara-Quechua women. The study identified several factors that may help predict these women's As methylation capacity, particularly ethnicity. Further studies are required on the mechanisms underlying these differences in As metabolism efficiency, and their importance for the risk of As-related health effects. Inefficient As methylation capacity has been associated with developmental delay in preschool children. Selenium has antioxidant and anti-inflammatory properties that protect experimental animals from chemically induced neurotoxicity and the results of a study designed to explore whether plasma Se levels affects As methylation capacity related to developmental delay in preschool children has been reported on[77]. A case-control study was conducted from August 2010 to March 2014 using participants recruited from the Shin Kong Wu Ho-Su Memorial Teaching Hospital in Taiwan. In total, 178 children with a developmental delay and 88 children without a delay were recruited. Urinary As species which included As^{III}, As^{V}, DMA^{V} and MMA^{V} were quantified using HPLC-HG-AAS whilst plasma Se levels were measured by ICP-MS. The results revealed that the plasma Se concentration was significantly inversely associated with the OR of developmental delay. Plasma Se concentrations were also positively associated with As methylation capacity; the percentage of iAs and percentage of MMA^{V} decreased, whilst the percentage of DMA^{V} increased. A high plasma Se concentration and high DMA significantly and additively interacted to decrease the OR of developmental delay; the OR and 95% confidence interval were 0.40 (0.18 - 0.90). This was reported to be the first study to show a combined dose-response effect of plasma Se concentration with As methylation capacity and developmental delay in preschool children.

Table 1 shows other applications of As speciation presented in the literature during the time period covered by this ASU.

Table 1 Applications of Speciation Analysis: Arsenic

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
AB, AC, As^{III}, As^{V}, DMA^{V}, MMA^{V}	ICP-MS	Sediment pore-water sample	Centrifuged, filtered (0.45 µm)	AEC, PRP-X100. Gradient (A)1.25 mmol L^{-1} Na_2HPO_4 + 11.0 mmol L^{-1} KH_2PO_4 (B) 2.5 mmol L^{-1} + 22.0 mmol L^{-1}	0.01 – 0.35 ng L^{-1}	Spike recovery	[78]
AB, As^{III}, As^{V}, DMA^{V}, MMA^{V}	ICP-MS	South African lichen *P. austrosinense*	Air-dried, stored in dark, ground, extracted with water (24 h)	AEC, PRP X-100, 17.5 mmol L^{-1} NH_4NO_3 in 1% MeOH, pH 8.6	MMA^{V} 0.1 µg kg^{-1}, not given for other species	BCR 482 lichen *P. furfuracea*), and INCT-TL-1 (tea leaf)	[79]
AB, As^{III}, As^{V}, DMA^{V}, MMA^{V}	ICP-MS, m/z 91 monitored ($^{75}As1^{6}O^{+}$)	Vietnamese rice	Air-dried, freeze-dried ground, 0.28 mol L^{-1} HNO_3, 95 °C, 90 min, centrifuged, filtered (0.45 µm)	AEC, PRP X100, gradient (A) 5.0 mmol L^{-1} $(NH_4)_2CO_3$, pH 9.0, 0.05% EDTA, 5% MeOH (B) 50.0 mmol L^{-1} $(NH_4)_2CO_3$, pH 9.0, 0.05% EDTA, 5% MeOH	0.5 – 2.9 µg kg^{-1}	BRC 211 (rice flour) and spike recoveries	[80]
AB, As^{III}, As^{V}, DMA^{V}, MMA^{V}	ICP-MS	Rice flour and oyster	extracted with 0.28 mol L^{-1} HNO_3, 95 °C for 90 min, centrifuged, filtered (0.45 µm)	AEC, Hamilton PRP-X100, isocratic 20 mmol L-1 ammonium phosphate, pH 6.0	Not given	NIST SRM 1568a (rice flour) and KRISS CRM (oyster tissue)	[81]
As^{III}, As^{V}, DMA^{V}, MMA^{V}	ICP-MS	Vegetables	Washed, diced, freeze-dried, MAE, 2% HNO_3, 90 °C, 17 min	AEC, PRP-X100, gradient (A) water (b) 30 mmol L^{-1} $(NH_4)_2HPO_4$, pH 5.8	0.07 – 0.08 µg L^{-1}	GBW 10047 (carrot), spike recoveries	[82]
As^{III}, As^{V}, DMA^{V}, MMA^{V}, Hg^{II}, MeHg, EtHg	ICP-MS	Lotus root	Homogenised, 5 mol L^{-1} HNO_3, UAE at 40 °C, 1 hr, centrifuged repeated, combined, neutralized filtered (0.45 µm)	RP-IP, C18 column, Gradient (a) 4.0 mmol L^{-1} TBAH (pH 6.0), (b) 4 mmol L^{-1} TBAH + 20 mmol L^{-1} L-Cys (pH 6.0)	As species 0.02 - 0.15 µg L^{-1}, Hg species 0.02 - 0.03 µg L^{-1}	NMIJ 7532-a (brown rice flour) and ERM-CE464 (mercury species in tuna fish), spike recoveries from five samples	[83]
As^{III}, As^{V}, phenylarsonic acid derivatives	ICP-MS	Lake waters	DLLME, pH 10, methyl-trioctylammonium chloride, octanol (extractant), methanol	RP-IP, C18 (4.6 mm x150 mm, 5 µm), isocratic 2.5 mmol L^{-1} sodium butanesulfonate and 10.0 mmol L^{-1} malonic acid, methanol/water (5 + 95, v/v). 30 °C	0.001-0.039 µg L^{-1}	Spike recoveries from three samples	[84]

			(disperser), centrifuged				

3.3 Chromium

Several *aspects of Cr speciation analysis have been reviewed*. The rather specialised topic of the application of magnetic nanostructures for preconcentration, speciation and determination of ionic Cr species forms the basis of a review with 113 references.[85] The graphic illustrating the principle shows a batch procedure in which one species is selectively retained from a large volume of sample solution on the surface functionalities of magnetic particles, which are retained (by a strong, externally applied magnetic field) while the rest of the sample is poured off (and possibly analysed for non-retained species). The retained component is released into a smaller volume, and thereby preconcentrated prior to analysis. A summary table contains 22 examples that are about equally divided between procedure in which Cr^{III} is retained and those in which Cr^{VI} is retained. Enrichment factors up to 250 were reported, with LOD values down to single-digit ng L^{-1}, for procedures in which quantification was by ICP-MS. Synthesis of the materials is described as simple and low-cost, and the procedure is promoted as rapid compared with “time-consuming column procedures,” which is a reasonable point because columns packed with NPs would exert a considerable back-pressure although this would be much less so with conventional SPE. The materials are perhaps not very robust and can only be re-used a limited number (5 - 10) of times. The reviewers cite nine previous reviews of the applications of magnetic nanoparticulate materials in chemical analysis and separations. A review (93 references) of recent advances in Cr speciation in biological samples opens with a tutorial introduction to the roles of Cr species in biological systems together with information about the concentrations in various compartments of living organisms.[86] Most studies so far have featured urine or blood (whole, serum, and plasma), but there are a few studies of saliva, tissue and joint effusion. A summary table contains 28 entries that includes information about the species, sample matrix, sample preparation, instrumental technique, and analytical merits. For 22 of these papers, a second table provides information on LOD, LOQ, linearity, range, trueness (possibly expressed as “recovery” of the certified value of the species in a CRM), and the CRM in question; though only 12 of the papers summarised report work validated by the analysis of a CRM. The reviewers highlight the capability of ICP-MS to perform ssIDA as a major advantage, but acknowledge that wider use is limited by the higher costs involved. There is a lengthy section on method validation in which researchers are chided for not giving this topic enough attention and for the lack of RM certified for Cr species content. These topics are reiterated in the concluding remarks, in which the difficulties of extracting Cr^{III} from proteins and of the possibilities of interconversion are also discussed. A retrospective by one research group (that of Jozsef Posta, University of Debrecen, Hungary) covers some 20 Cr speciation methods developed over the past 30 years.[87] Of the 44 references cited, 19 are to the work of this group, though not all of the articles are (a) about Cr speciation, (b) in the primary literature, or (c) in English. Methods featuring ICP-MS are only briefly mentioned, and so IDA is not discussed; all the methods mentioned are variations of SPE or LLE. The lowest LOD (20 pg mL^{-1}) was achieved by separation/enrichment on a C18 column with detection by nitrous oxide acetylene flame AES with hydraulic high-pressure nebulization. It should be noted that in the Web of Science entry for this article, all first names and last names are reversed.

To determine both the Cr speciation and isotopic composition in seawater, a method involving *coprecipitation with magnesium hydroxide* has been developed.[88] The report contains a tutorial introduction to the application of the isotopic fractionation that occurs in natural redox processes creating a distinct isotope signals in rocks, which thus provides a tracer that records Cr redox cycling, and allows deductions about the presence of oxygen in the geologic past. On addition of NH_4OH, only the Cr^{III} species out of solution (along with many other metals) is co-precipitated with MgOH. The solid was separated from the solution by centrifugation, washed and dissolved in 1% nitric acid. For the collection of total Cr, the Cr^{VI} in a separate sample was reduced (possibly surprisingly) by acidification with nitric acid to pH 1.8 and the addition of hydrogen peroxide (to a final concentration of 100 µmol L^{-1}). For isotope ratio measurements the sample volume (14 mL) was scaled up (along with reagents) so that at least 100 ng Cr was recovered. The Cr was determined by ICP-MS at m/z 52, with Sc as an IS, and recovery was monitored by the addition of a ^{50}Cr spike before precipitation. For the determination of isotope ratios by TIMS, the Cr^{III} was coprecipitated with ferric hydroxide, dissolved in HCl and passed through an anion-change column to separate iron as the anionic chloride; other cations were removed by passage through a cation-exchange column and the Cr loaded onto single rhenium filaments in a boric acid silica gel mixture. A weighed portion of a ^{50}Cr-^{54}Cr double-spike was added to the samples to correct for any isotopic fractionation that may have occurred during purification on the ion-exchange columns as well as during isotope ratio measurement in the MS. No contamination from the plastic storage vessels was detected, even after 2 y. The procedure has a preconcentration factor of about 5, producing an LOD of 0.04 nmol L^{-1}, (about 2 ng L^{-1}). The researchers found their MgOH procedure to be superior to methods based on Bi or Fe co-precipitation. Studies of the effects of UV irradiation and of the recoveries of Cr spiked as various organic complexes indicated that MgOH co-precipitation captured organically bound Cr within the dissolved total Cr fraction. The method was validated by the analysis of CRM NASS-6 (NRCC north Atlantic standard seawater) and spike recoveries, and applied to the analysis of seawater from a persistently anoxic fjord on the Pacific coast of Canada.

Several *dispersive microextraction methods* have been reported. Sieidi et al. devised a LLE procedure in which the dispersed solvent had a higher melting point than the sample solution and so was collected by freezing.[89] The procedure was selective for Cr^{VI} based on formation, at pH 2.0, of the complex with 1,5-diphenylcarbazone, which was extracted into the dispersed (by ultrasound) droplets of a deep eutectic solvent consisting of benzyltriphenylphosphonium bromide (BTPPB) and phenol. On cooling in an ice-bath, the solvent solidified and was sedimented by centrifugation. After melting at room temperature, the solvent was diluted (1:1) with MeOH and the Cr content determined by ET-AAS with pyrolitic graphite coated tubes (and no modifier). Full details of the furnace program are given. The optimum conditions were found by a central composite design strategy in which the parameters of pH, metal-to-ligand mole ratio, solvent volume and sonication time were set at 5 values. The pre-concentration factor of the optimised method was 34 and the LOD was 2 ng L^{-1}. The effects of other matrix metal ions and of ionic strength were investigated. The method was applied to the analysis of human urine and validated by spike recovery at two concentrations (25 and 50 ng L^{-1}); Cr at low double-digit ng L^{-1} concentrations was detected in all three samples. The sample volume was 4 mL and the analysis time was under 10 min, though no details of how many calibration standards were taken through the procedure is given. Yao et al. devised a procedure for the speciation analysis of waters in which both Cr species were preconcentrated prior to determination by ET-AAS.[90] To separate Cr^{III} by dispersive solid-phase extraction, 75 µL of a 2 mg/mL graphene oxide suspension was added to 10 mL of sample, adjusted to pH 7, and the solution agitated by ultrasound. The solution was filtered (0.22 µm) and then retained Cr washed off with several 70 µL portions of 0.5 mol L^{-1} nitric acid which were combined and diluted to 300 µL with MeOH. The filtrate was adjusted to pH 4 and 150 µL of 10% (m/v) Aliquat-336 and 120 µL of ionic liquid [1-octyl-3- methylimidazolium hexafluorophosphate][PF_6] were added and the mixture was ultrasonicated for 3 min. The resulting cloudy mixture was centrifuged when the ionic liquid phase settled at the bottom of the centrifuge tube allowing the upper aqueous phase to be decanted. Finally, the volume was adjusted to 300 µL with MeOH to decrease the viscosity of the ionic liquid phase. The procedure was optimised by a single cycle of the alternating variable search method, giving LOD values for both species of 20 ng L^{-1}. The method was applied to the analysis of six water samples, in two of which Cr^{VI} could not be detected, and validated by (a) the analysis of the same samples by a previously published dispersive LLME method, and (b) spike recoveries at 300 and 3000 ng L^{-1} from one well, one tap and one river water sample. The researchers point out that the method does not depend on changing the oxidation of one species (with subsequent total determination), does not require any chelating agents, organic disperser solvents, or highly toxic extraction solvents. To determine Cr^{VI}, Nafti et al.[91] selectively extracted Cr^{III} from alkaline digests of airborne inhalable dusts by a CPE method in which the Cr^{III} was complexed with 1-(2-pyridylazo)-2-naphthol. The surfactant was Triton X-114. The method was optimised by a single-cycle alternating variable search to give conditions under which all of the Cr^{III} was extracted leaving Cr^{VI} in the aqueous phase. The ET-AAS LOD was 0.1 µg L^{-1}. The authors write, in regard to validation by spike recovery, that accuracies were all lower than 7%. and recoveries were "99.8% for the Cr^{III} removal" and a "recovery exceeding 99.9% of Cr^{VI} extraction" was obtained, but there are no results presented to support these claims. Total Cr was determined directly on the acid digest of the filters. Samples from four different workstations were analysed for which the total Cr varied from 0.72 to 2.3 µg m^{-3} and the Cr^{VI} varied from 0.02 to 0.32 µg m^{-3}.

A *continuous LLE* technique has been devised for the separation and enrichment of Cr^{VI} from water samples.[92] The patented extraction device, which is described in detail in the paper, drew a stream of sample solution from a reservoir, with a volume of approximately 125 mL, through a 10 mL extraction vessel containing the extracting agent, methyltrioctylammonium chloride, in chloroform. After extraction, the Cr^{VI} in the chloroform phase, which was retained in the mixing chamber, was determined by ET-AAS. For method development, the sample was drawn through the apparatus by the suction of the nebuliser of the FAA instrument that was monitoring the Cr removed. The LOD was 0.01 µg L^{-1}. Total Cr was determined directly by ET-AAS. The method, which was validated by spike recoveries from water matrices that had no detectable Cr^{VI}, and by the analysis of the spiked samples by another method, was applied to the analysis of rain, tap, river and sea waters, all of which contained measurable concentrations of Cr^{VI} ranging from 0.093 to 1.42 µg L^{-1}. Total Cr varied from 0.43 to 2.32 µg L^{-1}. A potential problem is the rather long extraction times (getting on for 20 min) if the sample volume needed is around 100 mL. An LLE method based on extraction into a "tunable" solvent has been devised.[93] A tunable solvent is one that converts from a non-ionic form into an ionic liquid upon exposure to an atmosphere of carbon dioxide, and then reverts back to its non-ionic form when exposed to nitrogen gas. To separate and preconcentrate Cr^{III}, 8-hydroxyquinoline was added to the aqueous sample which was then mixed with the biphasic tunable solvent (preparation described), 1,8-diazabicyclo[5.4.0]undec-7-ene and decanol (1:1) while exposing the system to carbon dioxide. The resulting single phase was returned to the two phases by bubbling nitrogen while heating, and the aqueous phase separated leaving the Cr-oxine complex in the solvent phase from which it was then extracted into 0.25 mL of 1 – 2 mol L^{-1} nitric acid and the Cr determined by ET-AAS. A similar procedure was followed to separate Cr^{VI} based on the formation of the APDC complex. In both cases, the tunable solvent was recovered and reused. The method was optimised by the single-cycle alternating variable method, applied to the analysis of the aqueous extract of leafy vegetables and validated by spike recoveries (at single digit μg g^{-1} concentrations), interference studies, and by reduction of Cr^{VI} to Cr^{III} and the determination of total Cr via the Cr^{III} content. The solution LOD values were 0.05 and 0.07 μg L^{-1} for Cr^{III} and Cr^{VI}, respectively. Both species were detected in the extracts of six different vegetable samples at concentrations ranging from 0.8 to 3.6 μg g^{-1}.

An *HPLC-ICP-MS procedure has been described* in which Cr^{III} and Cr^{VI} were separated on an AE column (IonPac AS9-AC) by isocratic elution with a mobile phase of 0.55 mol L^{-1} nitric acid, without the addition of any chelating agents.[94] The separation took about 5 min with a flow rate of 1 mL min^{-1}. Although the method was described as optimised, very few details are provided. The slopes of the calibrations showed very clear compound-dependent responses, with that for Cr^{VI} being about 70% of that for $Cr^{III.}$ The method was validated by spike recoveries from tap and spring water at concentrations up to 100 μg L^{-1} and by the determination of total Cr in a spring water CRM (UME-1201, National Metrology Institute of Turkey). The LOD values were 0.09 and 0.03 μg L^{-1} for Cr^{III} and Cr^{VI}, respectively. No Cr species were detected in the water samples, but they were found in lettuce seedlings grown hydroponically and in extracts, o simulated gastric and intestinal fluids, of the plants. Lesniewska and Godlewska-Zylkiewicz[95] made a thorough study of the performance of an ion-pair reversed phase HPLC-ICP-MS method for the determination of Cr^{III} and Cr^{VI} in alkaline (0.1 mol L^{-1} sodium carbonate) extracts of soils. The method replaces the quadrupole ICP-MS instrument of a previously published method with a triple quadrupole instrument. The researchers investigated oxygen and helium, as reaction and collision gases, respectively, and chose helium because, although the background due to $^{40}Ar^{12}C^{+}$ was reduced by a factor of 400 with oxygen, compared to a factor of 150 with helium, the sensitivity was also decreased (by a factor of 20) due to the inefficient formation of $^{52}Cr^{16}O^{+}$. The species were separated in about 3 min on a C8 column (3 μm diameter particles, 4.6 mm × 33 mm) by isocratic elution with 1 mmol L^{-1} TBAH and 0.6 mmol L^{-1} EDTA at pH 7.2. The calibration curve slopes show that the response of Cr^{VI} was 96% of that of

Cr^{III}, and LOD values were between 0.08 – 0.09 µg L^{-1}. The method was validated by the analysis of two clay soil CRMs (CRM 041 and CRM 060 from Sigma Aldrich that contain 86 ± 3 and 195 ± 9 µg g-1 of Cr^{VI}, respectively), by spike recoveries from several soil samples, and by comparison of the slope of a standard additions plot with that of the regular calibration (no difference was observed). The researchers also developed an UAE procedure, thereby significantly decreasing the time needed. The paper contains a comprehensive introduction to the speciation analysis of Cr in soils, including a useful discussion of the problems of the determination of Cr by ICP-MS. There is also significant discussion of the possible effects of the relevant soil chemistry on the accuracy. Lorenc et al. applied procedures already published for multi-element speciation (As, Cr, Sb) by HPLC-ICP-MS to the analysis of flavoured bottled drinking water.[33] The Cr procedure was specific for Cr^{VI}, which eluted between 7 and 8 min in an AE HPLC separation with mobile phases of (a) 3 mmoL^{-1} Na_2EDTA and 36 mmol L^{-1} NH_4NO_3 at pH 4.6, and (b) the same composition at pH 9, applied in a 7-step "gradient" procedure, of which the last two steps appeared to be identical, namely 100% eluent A for 9.8 min. The column was a Hamilton PRP-X100 (4.6 mm × 150 mm). The paper contains limited information about the ICP-MS operation; the instrument contained a DRC with oxygen as the reaction gas. On the other hand, the paper does contain a very detailed account of the calibration and validation, including the uncertainty budget estimate. As it turned out, none of the five samples examined contained Cr^{VI} above the LOD of 0.1 µg L^{-1}, though three of the samples contained Cr^{III} and an unidentified Cr species. The paper also contains descriptions of the development of a SEC separation with ICP-MS detection (with which three Cr-containing peaks were observed), and of the application of a previously developed HPLC-ICP-MS procedure for As speciation. In addition, results were reported for an ES-MS/MS procedure in which samples were injected directly, though no further insights into the nature of the unidentified Cr species were obtained. To determine labile Cr^{III} and Cr^{VI} in natural waters, sampling by diffusive gradients in thin films which allows the determination of the time-integrated concentrations during deployment rather than the equilibrium concentrations at a particular time, has been employed.[96] Following elution of Cr species with 50 mmol L^{-1} Na_2EDTA at pH 9.5 the Cr species were separated in just over 4 min by AEC (4.6 x 50 mm, particle size 5 µm) with isocratic elution in a mobile phase of 40 mmol L^{-1} NH_4NO_3 at pH 7.4 and detected by ICP-MS at m/z 52. The instrument contained an octopole reaction cell with He as the collision gas. The introduction to the paper cites four previous studies to the determination of Cr species by HPLC-ICP-MS, but there are no citations in the method section, nor is there any explanation for the need to develop a new HPLC method. However, a full optimisation is reported that resulted in a procedure with LOD of 0.05 µg L^{-1} for Cr^{III} and 0.02 µg L-1 for Cr^{VI}. The calibration curve slopes show that the sensitivity for Cr^{VI} was 106% of that for Cr^{III}, a difference that may not be significant. The method was applied to a river water into which sewage was discharged and in which residents often swim. Although the title and abstract of an article by Caporale et al.[97] suggest that a new analytical method has been developed for the quantification of Cr^{VI} (in potentially contaminated soils from south Italy) by ID-HPLC-ICP-MS, it turns out that all the analytical methodology has been published previously. However, the article contains a useful critical discussion of the various procedures involved, particularly those concerned with extracting the soil samples. Problems with US EPA methods 3060A, 7196A and 7199 were encountered and discussed in terms of the organic matter in the soils, some of which was removed by a hexane extraction. The procedure involved spiking with $^{50}Cr^{VI}$, then extraction by focused microwaves (5 min at 80 °C) into 50 m mol L^{-1} EDTA + 5 m mol L^{-1} Na_2EDTA at pH 10, followed by AEC HPLC separation on a Ion pack AS9-HC anion-exchange column (4 x 250 mm x 9 µm) with isocratic elution, using the extraction solution diluted 1:9, as mobile phase. The spectrometer was in reaction cell mode with He as the collision gas.

A commercially available sample-handling device (prepFAST IC) that features a *low-pressure chromatographic separation of Cr species* has been evaluated for the analysis of various waters by ICP-MS.[98] The device is a flow-based microfluidic liquid handling system with computer-controlled syringe pumps and switching/injection valves, that allows a defined volume (300 µL) to be delivered to the anion-exchange column (Elemental Scientific 4 x 50 mm) by timed injection. Prior to injection, the sample was diluted online by a factor of 10 and eluted at 300 µL min^{-1} with a mobile phase of 2.1% ammonium nitrate at pH 1.9 containing 10 mg L^{-1} of Tm, which is described as a "chemical modifier." The results of the speciation analysis were compared with those obtained for Cr^{VI} by "conventional" anion-exchange HPLC of the undiluted samples with an Agilent column (4.6 x 30 mm) with a mobile phase of 5 mmol L^{-1} EDTA + 5 mmol L^{-1} $Na_2H_2PO_4$ + 15 mmol L^{-1} Na_2SO_4 at pH at 1.2 mL min^{-1} and a 100 µL injection volume. It appears as though two spectrometers were used, both of which were operated with He as the collision gas, but the authors speculated that better detection limits would have been obtained with ammonia. With the new method, both Cr species were eluted within 5 min, with baseline resolution, and Cr^{VI} was eluted before Cr^{III}. The LOD values were 7 and 12 ng L^{-1} for Cr^{VI} and Cr^{III}, respectively. A plot of the results for Cr^{VI} obtained by both methods for 40 samples and 10 QC standards, whose concentrations ranged from 0.09 to 32 µg L^{-1} had a slope of 0.9844 and an intercept of 0.2043 µg L-1. No statistical analysis was performed, so it is not clear whether these numbers are significantly different from 1 or 0, respectively. The researchers calculated that the average difference between the results for the samples was 1.9%, with the "syringe" LC results being higher. The authors pointed out the advantage of the much lower background for the metal-free syringe pumped system, compared with that for the HPLC method. The prepFAST IC device could also prepare a series of standards from a single stock and could be operated in a "total metals" mode in which a relatively large sample volume was introduced to the instrument in a carrier of 2% nitric acid.

Several *solid-phase extraction methods* have been reported for which the researchers synthesised the extraction material. A monolithic column SPME has been developed for Cr^{III} (and Sb^{III}).[29] Full details of the synthesis, by a one-pot co-condensation of carboxyethylsilanetriol Na salt (CES) and tetramethoxysilane, and characterisation of the monolithic material are given. The analytical columns were 5 cm lengths cut from a longer fused-silica capillary (530 µm id and 690 µm od). The analytes were extracted from 4.0 mL water samples at pH 4.5 flowing at 200 µL min^{-1} through the column, which was then washed with water and the retained species eluted with 150 µL of 10% nitric acid. The Cr and Sb contents of the eluate were determined by ICP-MS. Not all of the details of the experimental procedure are given in the paper (though there is supplementary information available, which provides some of the instrument operating conditions), though considerable details of the single-cycle alternating variable optimisation procedure are provided. The LOD values were 0.004 µg L^{-1} for Cr^{III} and 0.002 µg L^{-1} for Sb^{III}, low enough to detect both species in the three samples (lake water, rain water, and sewage) examined. The method was validated by interferences studies and spike recoveries at concentrations of 0.5 and 5.0 µg L^{-1}. Results were also presented for the Cr^{VI} and Sb^{V} contents, which were calculated by difference from the values for the total element content and those for the lower oxidation state. However, it was not clear how the total values were determined, presumably by direct analysis of the sample. In another study, involving synthesis and extensive product characterisation, a Cu sulfide nanocomposite material, selective for Cr^{VI}, was prepared.[99] Sample (30 mL pH 4.0) was pumped at 2.5 mL min^{-1} though a column (30 mm x 2.0 mm) containing 20 mg of the extractant, and, the retained Cr^{VI} eluted with 1.0 mL of 1.2 mol L^{-1} nitric acid at 2.0 mL min^{-1} and determined by AAS with an air-acetylene flame. For the determination of total Cr, any Cr^{III} was oxidised by heating with alkaline (pH 11.75) H_2O_2 at 80 °C for 1 h; the excess peroxide was removed by boiling for 8 min. The LOD was 0.15 µg L^{-1} with an enrichment factor of 30. Once again, the optimisation strategy, the results of which are in the supporting information, appeared to be a single-cycle alternating variable approach. There are no details of the calibration procedure. The method was validated by the analysis of two CRMs (GSB07-3174-2014 water, Institute for Reference Materials of SEPA, China and GBW10011, Geophysical and Geochemical Exploration Institute, China), by spike recoveries from both the water CRM, four water samples, and two flour samples in all of which Cr^{VI} was detected at concentrations ranging from 0.77 to 3.76 μg L^{-1} for the waters and from 0.059 to 0.128 μg g^{-1} for the flours and by interference studies. The most severe interferences were from arenite and arsenate, which could only be tolerated (± 5% error) at 2 and 2.5-fold excesses, respectively. The material was stable for at least 15 determinations. Some important experimental details are missing, e.g. the mass of sample taken for the microwave digestion of the flour sample and the final volume of the digest solution. In a report of the opposite approach (retention of Cr^{III} in a batch extraction method), more space is devoted to the synthesis and characterisation of the SPE material (a carboxylate-functionalized mesoporous silica) than is given to the development and validation of the analytical method in which the material is employed.[100] The results of the single-cycle alternating variable search optimisation of the analytical method, details of which are mostly given in the supplemental information, are scattered throughout the paper. It would appear that a sample solution (50 mL adjusted to pH 5.0) was placed in a 50 mL centrifuge tube that contained 10 mg of the solid phase extractant material. After mixing for 10 min, the phases were separated by centrifugation and the Cr^{III} in the supernatant was extracted into 2.0 mL of 1.5 mol L^{-1} nitric acid and determined by ICP-MS. Total Cr was also determined, presumably by direct introduction of the sample, and Cr^{VI} was calculated by difference. The LOD was 0.02 μg L^{-1}. There was no information about calibration, and the method was validated by interference studies and spike recoveries (at 0.3 and 0.6 μg L^{-1}) from three water samples (rain, lake and river); both species were detected in all three samples. No information on possible reuse of the material was provided. A similar approach was reported by Ali et al.[101] who synthesised and characterised poly-3-hydroxybutyrate-2-(dodecylthiocarbonothioylthio)-2-methylpropionate trimester, which was specific for Cr^{III}. A 50 mL sample volume (adjusted to pH 7.0) was aspirated though 100 mg of the material packed into a pipette tip (no details given) by manual withdrawal of the syringe plunger. The solution was then expelled through the tip, and the process, which took 5 min, repeated 5 times. The material was then extracted with 600 μL of 2.0 mol L^{-1} hydrochloric acid in a beaker, and the Cr determined by ET-AAS. Most of the instrument operating parameters are given, but there is no information about injection volume, furnace type or chemical modification. Total Cr was determined after reduction of Cr^{VI} with hydroxylamine hydrochloride (incomplete details). The LOD was 6 ng L^{-1}. The method was optimised by the single-cycle alternating variable method and validated by interference studies, the analysis of two CRMs (LGC -6010 hard drinking water and GBW-07605 tea), and spike recoveries (at 4 and 8 µg L^{-1}) from five different water samples (mineral, tap, lake, river and sea). No information about preparation of the tea sample was given. All five waters contained measurable concentrations of both Cr^{III} and Cr^{VI}. A SPE method in which both species were retained sequentially on the same (commercially available) adsorbent has been reported.[102] Both species were complexed with thiosemicarbazide (0.3 mol L^{-1}); but at pH 1 only Cr^{III} was retained, on the column (30 x 3 mm, glass) of PTFE beads whereas, at pH 7.0 only Cr^{VI} was retained. Samples were handled with a commercial FI system coupled to a FAA spectrometer with an air-acetylene flame. The loading and elution (0.5 mol L^{-1} nitric acid) flow rate was 5.0 mL min-^{1}. The loading times were either 60 or 120 s, but elution times are not given. Nor, unfortunately, was any information about the PTFE beads, other than the supplier was Sigma Aldrich. The method was optimised by the single-cycle alternating variable method, for which results are given in the paper. The LOD values were 0.13 and 0.14 µg L^{-1} for Cr^{III} and Cr^{VI}, respectively, based on a 120 s loading time. The enrichment factors, calculated from the ratio of the calibration slopes with and without preconcentration, were 61 and 56, respectively. The method was validated by interference studies, the analysis of a CRM (NIST SRM 1640a trace elements in natural water, which contains a total Cr concentration of 40 µg L^{-1}), and spike recoveries (at 20 µg L^{-1}) from four water samples (industrial and river), all of which contained measurable concentrations of both species that ranged from 5.4 to 54 µg L^{-1}. Readers of Japanese may be able to decipher relevant information from a report of another method in which the SPE material is commercially available.[103] In addition to an abstract and title in English, the tables and figures (including headers and captions) are in English. Two of the figures are flow diagrams of the procedure, in which the Cr^{III} in fly ash eluate is retained on Nobias Chelate PA1 resin treated with Fe^{III}. Any Cr^{VI} passed through and was determined by AAS. It was not clear how the retained Cr^{III} was eluted other than nitric acid was used. It also appeared that by controlling the pH at which the sample was loaded, selective retention was possible: Cr^{III} at pH 3.5 and Cr^{VI} at pH > 10.7.

3.6 Cobalt

Two papers have been published this year on the speciation of cobalt using two different methodologies: HPLC-ICP-MS and synchrotron X-Ray techniques. In the first paper HPLC-ICP-MS was employed to determine Co compounds in dietary supplements.[104] Separation was performed using an HPLC system equipped with a RP C8 column (21mm x 150mm, 5µm) and a gradient elution using ACN as mobile phase A and 8 mmol L^{-1} NH_4AC (pH=4.0) as mobile phase B flowing at 0.12 mL min^{-1}.

A membrane desolvation system was applied to remove the organic solvent load on the plasma. The procedure enabled the separation of six Co-containing species: Co^{II}, cyanocobalamin (CN-Cbl), hydroxylcobalamine (OH-Cbl) and the strongly adsorbed 5' deoxyladenosyl cobalamin (Ado-Cbl) and methylcobalamin (Me-Cbl). Co compounds were separated within 14 minutes and identified by retention time matching with available standards. The LOD values were in the range 0.007 to 0.031 ng Co mL^{-1}. The developed method was applied to two dietary supplements (multivitamin tablet and spirulina / chlorella tablet) as well as two locally purchased drinks. The Co species were extracted from the samples in less than 10 minutes using MAE and 0.5% (v/v) HNO_3 with recovery values within the range 94 to 100%. Results revealed that only Co^{II} and CN-Cbl were present in the drinks whereas Co^{II}, OH-Cbl , Ado-Cbl, MeCbl and an unknown compound were found in the extract of the spirulina/ chlorella tablet. Due to the lack of RMs, accuracy of the method was evaluated by conducting recovery studies in non-enriched drinks and supplements with two concentrations of mixed standard solutions. The recovery was in the range of 94 to 99% for all analytes. Moreover, the sum of the concentrations of individual Co species measured by HPLC-ICP-MS agreed well with the total concentration of Co obtained, after acid digestion, by ICP-MS. In the second paper the distribution and chemical state of Co and Ni in the hyperaccumulating tree (*Clethra barbinevis*) was evaluated by XRF and XANES.[105] The aim behind this study was to gain a better understanding of the mechanisms behind the tolerance of *C. barvinervis* to the presence of high amounts of these elements. Plants were hydroponically cultured with Hoagland solution and simultaneously exposed to 50 mmol L^{-1} of Ni and Co once per week for 3 years. Along with chemical analysis XRF spectrometry and XANES were employed to evaluate the distribution of Co, Ni and S in the adaxial leaf epidermis. The data obtained by XRF revealed that Co was present at the tip of the leaf (representing approximately 1/50 of the length of the entire leaf) whereas Ni was mainly distributed along the leaf edge. Results from XANES and chemical analysis showed that Co was mainly bound to sulfate whereas Ni to succinic and oxalic acid. In addition, a preferential accumulation of GSH was detected at the tip of the leaf which could favour the tolerance of this plant towards the presence of Co. The study concludes that the mechanisms governing the tolerance for Co and Ni are clearly different, *C. barbineris* differentiates Co and Ni and translocates them to the different parts of the leaf.

3.7 Copper

Copper is *an important nutrient as it is required as co-factor of several enzymes* in various organisms. However, excess of Cu generates reactive oxygen species (ROS) resulting in serious cell injury. Although several factors regulate Cu concentration in living systems, the accumulation of Cu is of particular concern in neonates. Tanaka *et al* [106] have investigated the variation in Cu distribution, with the aim to understand regulating factors, in serum, liver and kidney samples collected from neonatal rats (> 2 weeks old) and juvenile rats (5 weeks old). Liver and kidney samples were collected and homogenised with 50 mmol L^{-1} Tris-HCl at pH 4.0 whereas serum samples were separated from blood cells by centrifugation. Copper species from samples were analysed for by ICP-MS in combination with different size exclusion columns: for liver and kidney extracts a column with a cut off size > 800,000 Da was used (7.8 × 300 mm with a guard column) whilst for serum samples a column with an exclusion size > 300,000 Da (7.5 × 300 mm with a guard column) was used. In each case 50 mmol L^{-1} Tris-HCL at pH 7.4 at a flow rate of 0.6 mL min^{-1} was employed as eluent. The elution profiles of Cu in the different samples showed the presence of two major peaks which were assigned to superoxide dismutase 1 (SOD1) and metallothionein (MT) by retention time matching with available standards and also by measuring Cu to Zn molar ratio at each of the chromatographic peaks. Differences in the Cu concentration between neonatal and juvenile rats were detected. In neonatal liver the concentration of Cu was ten-fold higher than in juvenile rat livers. The accumulated Cu was bound to metallothionein to store Cu during the neonatal period and it was gradually released from metallothionein. In contrast, the amount of Cu bound to SOD1 increased with age. The physiological accumulation of Cu in neonatal liver was related to the low expression of the antioxidant protein Atox1 which reflects low oxidative stress in fetal and early neonatal periods.

Copper is also an *essential nutrient for plant development*. However, a high concentration of soil Cu can be toxic to plants, causing damage by ROS, photosynthesis inhibition and other mechanisms. In this sense, two papers *on the impact of Cu in the environment* have appeared this year. One of the plants most affected by the presence of high soil metal content is rice, as it grows in flooded soils, thus favouring the redox reaction and mobility of metals. Cui *et al*[107] explored the spatial distribution and Cu speciation from rhizosphere to rice grain of rice plants grown in mining-impacted padded soil. µ-XRF indicated that Cu was mostly present in rice root surface and epidermis rather than the xylem. µ-XANES evidenced the presence of Cu bound with C / N ligands such as alginate and histidine in roots, whereas thiol-S-bound Cu^{I} complexes, such as Cu^{I}-glutathione and Cu^{I}-cysteine, were detected in the root xylem. A paper that describes the influence of Cu on the photosynthesis process by using the anoxigenic purple bacteria *Rhodospirilium rubrum* as *in vivo* model has also been published.[108] The displacement of Mg and Cu in pigments (chlorophyll, Chl) and pigment–protein complexes (bacteriochlorophyll,l BChl) isolated form Cu-stressed cells was investigated using HPLC-ICP-MS. Bacteria cells were cultured in presence of different Cu concentrations (0.2, 2, 16 and 128 µmol L^{-1}) and collected after 4 days of incubation by centrifugation. The isolation of light harvesting 1(LH1) and reaction centre complexes (RCC) was performed using a protocol based on the sequential use of 50 mmol L^{-1} phosphate buffer (pH 7.0), 0.5% N-Ndimethyldodecylamine and 2% n-dodecyl-b-d-maltoside. Pigments, including RC and LH1 complexes, were then extracted with acetone. Proteins were separated by SEC on three 10 x 300 mm columns connected in series with an aqueous buffer as the mobile phase. Pigments were separated on a C18 column with a MeOH:ACN gradient. Metal- EDTA complexes and standards of Mg-Chl a, Cu-Chl a and Cu BChl were employed as calibrants to quantify the metal concentration in the proteins and pigment extracts. These analyses, along with results from UV/Vis/NIR and fluorescence measurements, revealed a strong inhibition of the photosynthetic reaction centre at 2 mmol L^{-1} Cu^{II} which was related to the formation of Cu and Mg containing BChl degradation products which are unsuitable for performing photosynthesis.

Finally, the combination of *µ-XRF and µ-XANES analysis has been employed to investigate the influence of Cu distribution and speciation* on the colour of the Chinese underglaze copper-red on porcelain from the Yuan Dynasty.[109] Differences were observed among the coloured areas of the porcelain. µ-XANES evidenced the occurrence of Cu^0 particles in black regions whereas in the red coloured region Cu was present as Cu^I and Cu^0. The authors attributed the presence of Cuº particles and their size as the main factors responsible of the different colours in the porcelain. Moreover, XRF measurements showed a strong positive correlation among the distribution of Cu, Pb and Zn in the black coloured region suggesting Zn and Pb as promoters of the formation of metallic copper particles. Although the study can be considered preliminary it clearly shows the potential of synchrotron X-ray techniques for evaluating the technology behind historical artworks as ceramics.

3.8 Gadolinium

A very comprehensive Perspectives paper, in the journal Metallomics, discusses the *speciation and biological fate of gadolinium containing MRI contrast agents* in patients undergoing scans.[110] This has become a controversial issue as their use has recently been shown to have unwanted clinical side-effects, leading to the requirement by certain regulatory agencies for a risk-benefit analysis prior to use for an individual patient's MRI scan. The review poses questions related to requirements for Gd-speciation *in vivo* and identifies the analytical and spectroscopic methods that can be used to determine the biological distribution and chemical speciation of the residual Gd left for up to 5, and in some cases more, years in the patient. The review covers a number of non-destructive as well as destructive methods, including: unenhanced T_1 weighted MRI, NAA, and XRF, used for *in vivo* studies; synchrotron XRF, SEM-EDX, EXAFS, LA-ICP-MS, MALDI-ToF-MS and NMR, used for tissue sections; and HILIC-ICP-MS and GPC-ICP-MS, used for tissue extraction samples. However, only the latter three methods were able to provide any indication of the actual speciation of Gd, rather than the atomic environment of the Gd atom i.e. it's binding partners, which whilst being of some qualitative use these methods leave a lot of the required speciation information unknown. The HILIC method was used to investigate the Gd speciation in skin biopsy samples from a patient with nephrogenic systemic fibrosis (NSF). The soluble species from the skin sample were extracted in water:ACN (4:1) for 72 hr, but some doubts were expressed in relation to the effectiveness of the reagents used. Intact Gadoteridol was detected in the sample 8 years after administration as a contrast agent, but the paper states that it was clear that the soluble species extracted represented only a small proportion of the Gd present, the most important being insoluble $GdPO_4$ somehow detected and identified by LA-ICP-MS. The most interesting aspect of the review was the use of MALDI-ToF-MS which as a molecular MS method can easily highlight any Gd-containing species due to the specific isotopic pattern related to Gd, which has 6 stable isotopes. Molecular MS-imaging was used to detect Gd contrast agents in mouse liver and in myocardial infarcts. However, the use of the MALDI matrix could be considered as analyte extraction, so the detected Gd-species might not be representative of those present in the sample. Additionally, quantification could be inaccurate, as the desorption and ionisation differ between Gd-containing species. As with most approaches experimental rather than analytical control materials must be prepared to validate the results. As always however, the challenge is to determine the chemical form without interfering with the species or disturbing the equilibria established in the human body. The authors conclude that a combination of all these approaches is likely to yield the most information, but that despite the increasing number of studies the actual biological distribution and speciation of the residual Gd are not fully established.

New *bioimaging approaches for the investigation of Gd-containing contrast agents in biological tissues* have been reported. In a paper not available for inclusion in the perspectives paper, the group of Linscheid used molecular and elemental MS in a complementary way, to investigate the imaging of a Gd-contrast agent, Gadovist in mouse brain, heart, kidney and liver tissues.[111] Whilst this is by no means the first complementary use of these techniques, which the authors attribute to publications in 2009, it is an important contribution to the literature in the area as it demonstrates some of the technical difficulties of combining these methods, and also the strengths and weaknesses, that make them complementary. In the final developed work-flow the tissues were snap frozen, cryosectioned, placed on glass slides and then dried in a desiccator before application of dihydroxybenzoic acid (DHB) (20 g L^{-1}) made up in ACN:water:trifluoroacetic acid (60:40:0.1, the matrix) . Matrix application was performed in the range of 15 to 30 cycles onto the thin sections using an airbrush, with a 30 min drying period between matrix coats. The drying prior to preparation and between matrix application helped to maintain spatial resolution of the cellular organelles in the MALDI analysis. The thin sections were analysed in a MALDI Orbitrap XL instrument equipped with a nitrogen UV laser (337 nm), in positive ion mode using a mass resolution of 60,000 and measurement of ions related to the free ligand (BT-DO3A) at m/z 451.240 and the ligand bound to Gd at m/z 606.141. Both signals were normalised to that for the DHB matrix signal at m/z 273.039, which was required due to the significant drop in signal over time, thought to be due to laser stability, matrix sublimation, extraction efficiency, ionization efficiency, ionization suppression, detection efficiency, multiple charge states, and ion stability. Following MALDI analysis the same sample was printed with ^{165}Ho spiked ink using a modified ink-jet printer to act as an internal standard (IS) and then subjected to LA-ICP-MS analysis using a double focusing SF-ICP-MS instrument operating in low resolution mode. A defined sample area was ablated line by line with a focused Nd:YAG laser beam operated at 213 nm tuned for complete ablation of the tissue sample. The MALDI-MS/MS spectra were identical to those of the pure compounds and collision induced dissociation experiments confirmed that the observed m/z signals belonged to Gadovist and the free ligand. The spatial distribution of the contrast agent in the heart, kidney and liver tissues reflected the location of blood vessels. No signal was detected in the brain sections analysed which was shown by the LA-ICP-MS analysis to be related to the low concentration of contrast agent present. The lower LOD achieved using LA was the main advantage over MALDI, whereas the molecular analysis showed that the signal intensity for the free chelate was greater than the Gd-containing complex. The paper did not speculate on whether this finding related to loss of Gd due to the analytical step or if it reflected a biological process, but if the intensity of signal is also reflected in the concentration of each then it is interesting to speculate whether this relates to the release and retention of Gd observed in patients with NSF. Analytically the MALDI application was more technically difficult and showed significant signal drift over the course of the analytical cycle. However, the authors showed that the novel use of ink-jet printing the IS onto the tissue sample, whilst not perfect, represented the most suitable approach for normalisation compared to the others investigated during the work. The use of LA-ICP-MS/MS compared to using a single quadrupole instrument to investigate Gd in skin and brain tissues has been investigated.[112] For MS/MS analysis Gd and P were monitored with an oxygen mass-shift of +16 m/z, whereas Ca, Fe and Zn were measured on mass. For all elements the LOD values using MS/MS were lower, with P being determined using a liquid sample introduction single quadrupole ICP-MS as it was not possible to measure this effectively otherwise. Quantification of Gd and the determination of LOD values of the elements of interest were performed by in-house prepared matrix-matched standards consisting of homogenised lamb brain spiked with respective elements and mounted in thin tissue sections with a thickness of 20 µm on glass slides. The concentration of the elements in the matrix-matched standards was established after digestion with H_2O_2:HNO_3 (1:4 v/v) and using conventional ICP-MS analysis. An ArF excimer laser emitting nanosecond laser pulses at 193 nm was adapted to generate squared pixels, but unlike the previous publication the use of an internal standard was not mentioned, so it is unclear whether signal drift was a problem as with the former work.

3.9 Gold

There are three papers covering *the speciation of Au, all concerning NPs*, to report on this year. The first of these reviews the literature concerning analytical methodologies for studying the cellular uptake, processing and localisation of gold nanoparticles[113]. The section covering alterations in "chemical state", defined as the chemical nature of the surface of the AuNPs, contains five references which could be termed elemental speciation. The authors mention the potential disadvantages in the use of HPLC with NPs, the potential loss of analyte integrity due to the mobile phase composition (although it would hoped that workers have verified that this would not be the case via batch experiments for example) and the often incomplete recovery of NPs from analytical columns and suggest that CE is a more appropriate separation technique for NPs. A study has shown that citrate-stabilized Au nanoparticles (40 nm nominal diameter), injected into Wistar Rats, undergo degradation either during transport to or within the liver tissue.[114]. In this study, liver samples were analysed using spICP-MS, TEM and HPLC-ICP-MS. The spICP-MS analysis revealed the presence of the originally administrated Au NPs and, due to the elevated baseline compared with that obtained for Au NP standards, that some NP dissolution had also occurred. Images obtained using TEM showed the predominant presence of particles of significantly smaller diameter (6 ± 2 nm) than those administered. These were not detectable using spICP-MS as the particle size LOD was 18 nm, presumably due to the elevated baseline from ionic Au. Subsequently, liver lysate samples were injected onto a C18 column (1000 Å pore size, 250 x 4.6 mm) and separated in seven minutes with a mobile phase of 10 mmol L^{-1} ammonium acetate, 10 mmol L^{-1} SDS at pH 6.8 flowing at 0.5 mL min^{-1}. This approach confirmed the presence of two different size NP fractions previously detected and, additionally, an unidentified low-molecular mass species. These findings, as the authors also conclude, underline the need for various analytical techniques to be used if the fate of NPs in biota are to be fully understood. A separate group of workers have studied the uptake and transformation of Au^{III} and Au NPs in a green algae.[115] The algae (Desmodesmus subspicatus (Chodat) Hegewald et Schmidt (SAG Strain No. 54.80)) were cultured in Bold's Basal Medium (BBM), extracted and then suspended in either high purity water (HPW) or filtered river water to which was added the ionic or NP Au to give a final concentration of 50 µg L^{-1}. After a suitable sample preparation procedure quantification of the total Au content in algal cells, supernatants and wash solutions was by ICP-MS and Au species by HPLC-ICP-MS. These separations were performed using a PLRP-S400 column (8 µm particle size, 400 nm pore size, 150 mm x 4.6 mm) with a mobile phase of 10 mmol L^{-1} SDS and 5% MeOH flowing at 0.5 mL min^{-1}. The HPLC-ICP-MS LOD values were: 2.2 ng L^{-1} for Au^{III}, 2.8 ng L^{-1} for 10 nm Au NPs and 3.7 ng L^{-1} for 40 nm Au NPs. Significant losses of Au^{III} and Au NPs were observed in BBM, HPW and river water and a detailed discussion of the possible causes of this is given. Algal uptake of Au^{III} was typically 24 µg g^{-1} and it was observed that uptake of Au NPs decreased with a decrease in nominal particle size, typically 20 and 12 µg g^{-1} for 20 and 10 nm NPs respectively. After extraction from the algal cells, suspension in in 10 mmol L^{-1} SDS solution (5 mL) and sonication using an ultrasound probe for 15 min, and analysis by HPLC-ICP-MS it was found that Au^{III} was converted into intracellular AuNPs of 6 – 7 nm in size. The 10, 20 and 40 nm AuNPs were not found inside the algal cells but their hydrodynamic size increased due to the formation of a biocorona. No effect on algal growth was noticed during the 24 h incubation period.

3.10 Halogens

A number of *new methods for the measurement of I and I-containing species in environmental matrices* have been investigated, both to determine what I-containing species are present in high salt containing brine solutions and in drinking water samples after treatment, and also to determine the stability of I-species in soil extracts and to validate new extraction methods for soil samples. Each of these four approaches uses HPLC coupled to ICP-MS or ICP-MS/MS. Taken together this body of work shows that some consistency in the types of LC approaches used for the determination of I speciation with ICP-MS as the detector, is becoming apparent, with AE prevalent for inorganic I separations and SEC to investigate the binding of I to organic moieties. For AE the columns used were of the same type, an IonPac AS-14 column containing a resin based macroporous bead consisting of ethylvinylbenzene crosslinked with 55% divinylbenzene and an AE layer functionalized with quaternary ammonium groups. This column was used both for water and soil extract samples, whereas for the separation of larger I-containing species there was no consistency in the type of SEC column used. Cui *et al*[116] investigated the speciation of the inorganic I species iodate and iodide in high salt brine solutions using the aforementioned AE column. The main problem they observed was peak splitting when analysing samples containing a high salt concentration matrix, which was overcome by optimisation of the eluent containing ammonium carbonate at pH 10. Linear standard calibration plots were obtained in a concentration range of 1.0 - 100 $\mu g\ L^{-1}$ as I for both iodate and iodide and the LOD values were 0.05 $\mu g\ L^{-1}$ for the former and 0.20 $\mu g\ L^{-1}$ for the latter. The method was used for the analysis of primary high salt brine and dechlorinated light brine from a chlor-alkali plant. The changes in I speciation during the different stages of water purification in a public water treatment plant have been investigated.[117] A multi-mode SEC (Asahipak GS-2G 7B) column coupled to ICP-MS was used to investigate the large iodinated humic and fulvic acid compounds present in the untreated water and the inorganic I species produced during the treatment process were monitored once again using the IonPac AS-14 AE column mentioned above. The humic / fulvic acid compounds were characterised off-line using fluorescence spectrometry with multivariate analysis. No toxic I-containing disinfection by-products were detected at any stage of the treatment process using these approaches and the method showed that treatment with ozone was effective.

Whilst there have been a number of studies looking at I speciation in liquid samples, such as freshwater, seawater and urine, which mostly involve straightforward 'dilute and shoot' sample preparation, more complex methods are required for solid matrices such as soil. Two recent papers on the analysis of I species in soil both focus on *the sample preparation methods that can be used and their effectiveness at maintaining the correct species during sample preparation*. In the first an alkaline extraction was used initially, but it was determined that 0.5 mmol L^{-1} EDTA was required to maintain the I species, particularly as heating to 150 °C for 3hrs was required, which was the shortest time possible to extract the species but maintain their stability.[118] The analytical system once again used the IonPac AS-14 column coupled to ICP-MS to separate the species with good peak shape. The method was applied to several spiked soil reference materials providing recoveries of IO_3^- and I^- ranging from 85 to 115%. In real soil samples from an I rich area of China no IO_3^- was detectable with only I^- present and species recoveries were 86 - 90% compared to the total I present. The authors commented that the extraction method may have destroyed the association of iodine with organic materials but did not fully investigate this aspect. The second paper on iodine speciation in soil solution investigated a more ambitious approach involving microdialysis to maintain the original species present in the sample.[119] Microdialysis is an emerging technique that has previously been used for the *in situ* and minimally invasive sampling of soil solution to assess the prevalence and composition of nitrate, ammonium and amino acids in some agricultural soils. The technique has the potential to maintain the species composition as well as provide information on the spatial and temporal resolution of the soil solution chemistry. In this study, the microdialysis system consisted of a syringe pump, equipped with four syringes to deliver the perfusate solution. The syringes were attached, using adaptors, to a microdialysis probe (CMA 20/Microdialysis AB, Stockholm, Sweden) with a polyethersulfone (PES) membrane (10 mm long, 500 mm outer diameter with a 100 kDa molecular weight cut-off (MWCO)) and used a small volume vials (0.5 mL) for dialysate sample collection. In order to assess their sampling suitability, the permeability factors and effect of perfusion flow-rate on I^- and IO_3^- recovery were examined *in vitro* using stirred solutions. The experimental set-up was used to sample native soluble I at a range of water contents and I-enriched soils to investigate I soil dynamics. Total I concentrations were measured using ICP-MS. The iI species were determined by AE using a PRP X-100 column and the molecular weight distribution of organically bound I was determined using an AdvanceBio SEC 130 Å SEC column coupled to an ICP-MS. The most effective recovery rates in stirred solution were observed with the slowest perfusion flow-rate yielding 66% and 71% for I^- and IO_3^-, respectively. The use of SEC coupled to (i) UV and (ii) ICP-MS analysis provided detail regarding the molecular weight distribution of dissolved organo-I compounds, which were detected with approximate molecular weights between 0.1 and 4.5 kDa.

3.11 Iron

This year the *research interest in Fe has been primarily focused on its use in nanoparticulate* form. Iron-based NPs, mainly Fe oxide, have several uses in medicine, for instance as a contrast agent in MRI, and in nutrition as a supplement to prevent Fe deficiency. However, there are still concerns about their safe use. The first study reviewed here describes the characterisation of ferumoxytol, a suspension composed of Fe-oxide NPs used as an MRI contrast agent, in biological matrices such as rat blood cells and plasma. For NP characterisation, a plethora of techniques were applied including TEM, AF4-UV-MALS-ICP-MS/MS and sp-ICP-MS.[120] Rat blood cells and plasma were spiked with ferumoxytol to give 0.150 mg mL^{-1}. Afterwards, the spiked samples were submitted to different treatments depending on the applied technique. Regarding TEM observations, ferumoxytol- spiked samples were centrifuged, fixed onto circular copper films and air-dried at room temperature. For spICP-MS measurements samples were again centrifuged and the supernatant was then spiked with ferumoxytol prior to dilution. The results provided by the different techniques clearly evidenced the important effect of the sample matrix on Fe-oxide NP size distribution and morphology. Images by TEM showed that the initial morphology (spherical) and size distribution (18 ± 5 nm) was altered in blood samples where the NPs became polydisperse (114 ± 14 nm) with a rod-shaped morphology. The spICP-MS analysis of plasma samples provided a particle size of 52 ± 1 nm, suggesting the formation of NP aggregates. In the cell fraction a mean particle diameter of 33 ± 2.0 nm was found with a monodisperse size distribution similar to that of pristine nanoparticles. The differences observed between the techniques were attributed by the authors to both the low specificity of TEM and the fact that size distribution calculations by spICP-MS are based on the presence of nanoparticles of spherical geometry. Discrepancies in NP size distributions were also found between spICP-MS and AF4-MALS. The size overestimation by AF4-MALs was attributed to factors such as the presence of surfactants in the mobile phase of AF4, the presence of a hydration / corona layer on particles in solution and possible dissolution of the NP carbohydrate coating. The results clearly showed that NP characterisation in biological systems is challenging and requires the application of different but complementary techniques. In a separate report, Fe-based nanoparticle metabolisms and bioavailability in rats was evaluated. [121] Rats were fed a milk formula fortified with isotopically enriched Fe-oxihydroxide NPs, $^{57}Fe^{III}$-NPs, and $FeSO_4$. After two weeks of feeding, rats were sacrificed and liver and blood samples obtained. A speciation quantitative methodology consisting of HPLC-ICP-MS followed by post column IDA with a ^{57}Fe isotopically enriched standard was applied. Different separation columns were employed depending on the type of sample: An AE column for serum, a cation exchange column for erythrocytes and a SEC column for liver samples. The resulting mass flow chromatograms evidenced similar contents of Fe among the groups of rats that received fortified and non-fortified formula milk regardless of chemical specie of Fe administered. In contrast, the Fe content associated to transferrin, the major serum Fe transport protein, varied significantly among the groups under study. The highest serum Fe concentration was found in those rats that received fortified formula milk. The use of isotope pattern deconvolution allowed the authors to differentiate between the endogenous (natural) and exogenous (^{57}Fe) Fe content present in each of the Fe specie. These results showed that the labelled NPs were incorporated to a higher degree (> 50%) than $FeSO_4$ which is the most commonly used Fe fortifier. Based on the results obtained the authors postulate the use of Fe NPs as a good candidate for Fe fortification. However, the current EU directive governing the use of metal-based NPs as food additives is quite restrictive due to safety concerns. The FDA has approved six types of Fe-carbohydrate NPs to treat Fe-deficiency anaemia although the approval of additional Fe-carbohydrate NP drugs is hindered by the absence of robust and accurate methods. The current methods for quantifying Fe release from Fe NPs involves the measurement of the total Fe (TI), transferrin-bound Fe (TBI) and non-transferrin bound Fe (NTBI). The NTBI is associated to the Fe bound to ferritin, albumin and small molecules as citrate. In the third paper discussed here a method based on HPLC-ICP-MS for the direct determination of Fe NPs in plasma along with other iron species, such as TBI, PBI (ferritin and albumin) and LI (labile Fe, including Fe citrate) was developed.[122] Human plasma samples were spiked with a nano-Fe drug (Ferrlecit). The samples were then injected onto two SEC columns in series: a 3 µm, 300A, 4.6 x 50 mm column followed by a 3 µm, 300A, 4.6 x 300 mm column using a mobile phase of 10 mmol L^{-1} TRIS-HCl (pH 7.4) flowing at 0.4 mL min^{-1}. Four main Fe containing peaks were observed with elution times ranging from 10 to 16.5 min with the intact FE NPs eluting at 9 min. With the aim of stablishing the identity of Fe species present in the plasma, a calibration curve with known protein standards was employed to estimate the molecular weights of the Fe containing peaks. The first peak was associated with Fe NPs, followed by ferritin, albumin, transferrin and finally Fe species containing citrate. The identity of the Fe containing peaks was confirmed by MALDI-MS or ES-MS/MS. Of special relevance was the identification of the low molecular weight compounds found in the LI fraction. The main species identified were Fe citrate complexes such as $[Fe_3(Cit)_3H]_2^-$, $[Fe_2(Cit)_2H]^-$ and $[Fe_3(Cit)_3H_2]^-$. The developed method was validated following the FDA guidelines. The linear range for DBI was 0.3-50 mg L^{-1} whereas for TBI, PBI and LI the range was 10-1400 mg L^{-1}. The LOD values were determined as 0.3 mg L^{-1} for DBI and 10 µg L^{-1} for TBI, PBI and LI. Due to the lack of reference materials, the accuracy of the method was evaluated by conducting recovery studies in human plasma and the recovery of all analytes was in the range of 83 - 113%. The method was further applied to blood samples of healthy volunteers administered with 125 mg of Fe nano drug. Results showed the presence of DBI, TBI and LI in blood samples. The proposed method enabled the direct determination of Fe-carbohydrate drugs in clinical samples while simultaneously measuring the iron species released from nanoparticles.

Similarly, *a method to determine directly non- transferrin bound iron* (NTBI) in rat plasma based on the use of SEC-ICP-MS has been developed.[123] Animals were supplemented with $^{57}Fe^{II}$-ascorbate by means of a feeding tube inserted into the stomach. Blood samples were removed before and after supplementation by means of a series of catheters surgically installed into the portal vein, caudal vena cava and cranial vena cava. These were analysed for low molecular weight (LMW) and high molecular weight (HMW) compounds. For the former, two Superdex Peptide 10/300 GL columns connected in series were employed whereas for HMW compounds a Superdex 200 10/300 GL column was used. The mobile phase for both chromatographic set-ups was 20 mmol L^{-1} NH_4HCO_3 at pH 8.5. The resulting eluent flowed into the ICP-MS and the ^{56}Fe and ^{57}Fe signals monitored. Several low mass iron complexes were detected but none of them originated from ^{57}Fe enrichment but rather from internal Fe stored within the body, perhaps as macrophages. The absence of NTBI in the blood samples was attributed to two factors: 1) adsorption of NTBI compounds to the chromatographic columns employed and 2) the low level of transferrin saturation (from 8 to 60%) in the blood samples as NTBI only is detected in plasma with transferrin saturation values higher than 60%.

Two papers describe *the speciation of Fe in environmental samples*, one of them with the novelty of incorporating 3D printing technologies. This technology was employed to manufacture a SPE microcolumn for Fe preconcentration from environmental waters followed by ICP-MS measurements.[124] The microcolumn consisted of an acrylate resin stacked by interlacing cuboids having a square cross-sectional area. The dimensions of the packing were limited by the resolution of the 3D printer. The best analytical performance was obtained with the use of a microcolumn consisting of 30 layers of interlacing cuboids (2.7 x 0.3 x 0.3 mm length, height, width). The experimental set-up included two fabricated microcolumns (C1, C2), five-eight port valves and three peristaltic pumps. All valves were connected to an interface and computer controlled. The analytical protocol included the following steps: 1) Total Fe determination by loading the samples (pH 5.0) into C1 followed by the application of an air-stream for the removal of sample matrix and 2) Fe^{III} determination by loading the samples (pH 4.0) into C2 followed by the application of water for the removal of sample matrix and weakly adsorbed Fe^{II}. The adsorbed iron species were eluted with 2.5% HNO_3 and transported to the ICP-MS. Under optimal conditions, LOD values of 1-2 ng L^{-1} were obtained. The accuracy of the method was validated by analysing the CRMs NRCC SLEW-3, and NIST 1640 a and 1643e which have certified values for total Fe content of 0.568 ± 0.059, 36.8 ± 1.8 and 95.7 ± 1.4 µg L^{-1} with the found values being 0.571 ± 0.003, 38.0 ± 8.7 and 94.7 ± 0.3 µg L^{-1}, respectively. The method enabled Fe speciation to be performed in a simple, cheap fashion without the need for pre-oxidation or pre-reduction steps. The second paper covers of Fe^{II} and Fe^{III} speciation in magmatic hydrothermal fluids using a combination of synchrotron-based XRF and

XANES analysis and Raman spectroscopy.[125] Different experimental variables were taken into consideration: 1) mineral assemblage, including hematite-magnetite (HM), magnetite pyrite-pyrrhotite (MPP) and fayalite-magnetite–quartz (FMQ); 2) chlorinity, including chloride added in the form of HCl, NaCl, ferrous or ferric chloride and 3) temperature and pressure up to 600 °C and 885 MPa. Solubility of the Fe was higher in oxide-silicate assemblages than in sulfide bearing assemblages. The XANES results showed Fe^{II} was the predominant specie in all experiments with $FeCl_x(H_2O)_{2-x}^{6-x}$ (where x = 0-3) and $FeCl_4^{2-}$ or $FeCl_3(H_2O)$ for low and high Cl-Fe ratios, respectively. Ferric ion in solution showed a transition from $FeCl_2(H_2O)_4^+$ at temperatures > 100 °C to $FeCl_4^-$ between 100 and 200 °C with $FeCl_4^-$ remaining the most abundant species at 600 °C. Data from Raman spectra were in agreement with those provided by XANES. The results obtained provided new information and insights into the solubility and speciation of Fe in hydrothermal solutions over a wide range of conditions of special relevance to hydrothermal systems.

3.12 Lead

Two studies of *Pb speciation (by X-ray spectrometry) in respirable dust* have been reported. In the first the absorption kinetics of Pb from indoor dust, collected in Port Pirie, South Australia (home to one of the largest primary Pb smelters in the world), following a single dose instillation into C57BL/6 mice was followed by XRF microscopy, and the influence of mineralogy on Pb absorption and particle retention was investigated with XANES.[126] According to the article, Pb speciation in the dust was determined (by EXAFS) at the Materials Research Collaborative Access Team beamline 10-ID at the advanced photon source of the Argonne National Laboratory. But according to the supporting information, it was soil that was analysed at this facility, whereas the particles in the lungs were analysed at the X-ray XRF microscopy beamline at the Australian Synchrotron, Melbourne. The Pb speciation in the dust was reported, but it is not clear where this analysis was conducted. The researchers found that 44% of the Pb was bound to organic matter, and the remainder of the Pb species comprised hydrocerussite ($Pb_3(CO_3)_2(OH)_2$, 21%), galena (PbS, 11%), Pb-phosphates (chloropyromorphite, $Pb_5(PO_4)_3Cl$, 10%) and mineral-sorbed Pb (Pb oxides and Pb adsorbed onto clays, 13%). In the second study, the particles were collected from both a residential and industrial site in Greater Cairo, Egypt and Zarqa, Jordan.[127] The X-ray absorption spectra at the Pb L3-edge (13.039 keV) were measured at the XAFS beamline of the Elettra Synchrotron Radiation Facility (Trieste, Italy), with an average storage ring current of about 160 mA, ring energy 2.4 GeV and photon flux ranging from 10^9 to 10^{10} photon s^{-1}. The results showed that, when compared to the spectra of 13 Pb compounds, chosen to cover all the expected Pb species, the dust from both the industrial site in Greater Cairo and an urban site in Zarqa contained Pb^{II} and Pb^{IV}, whereas that from a residential area of Greater Cairo contained only Pb^{II}.

3.13 Mercury

Following the pattern of recent years there is still a strong interest in mercury speciation and this topic has been *reviewed with respect to the analysis of sediments* for different Hg species.[128] The review, which cites 230 papers, gives a brief overview of the environmental chemistry of Hg before considering six different areas: storage and pre-treatment of samples, reference materials, extraction methods, pre-concentration procedures, the separation of Hg species and finally detectors. It is good to see method validation with the use of appropriate CRMs at the beginning of the review, which contains a table of available suitable CRMs, and the authors point out that if possible not only should the matrix be matched but also if possible the Hg species mass fraction as well. This is so that artefact formation, which may occur due to the extraction procedure used, can be more readily assessed. They also advise against the use of RMs which have been spiked with Hg species as the extraction efficiency obtained from materials prepared in this way is likely to be greater than that obtained for natural materials. The section on analyte extraction from sediments gives a detailed overview of these procedures and highlights some of the problems that may occur, such as incomplete extraction and artefact formation, and also includes a brief discussion on the use of X-ray based techniques for which no analyte extraction is needed. The pre-concentration section discusses both solid phase and liquid / liquid based techniques whilst both chromatographic (Ce, GC and HPLC) and non-chromatographic (hydride generation and thermal desorption) are comprehensively covered in the separation section. The authors do state that for GC the Hg species need to be derivatised into a volatile form which is not always the case as there have been reports of methods of the direct injection of un-derivatised Hg species onto a GC column, often with pyrolysis-AFS as the detector. Finally, all the main detection techniques, which are mainly AFS and ICP-MS are covered in which the authors appear to be recommending HPLC-ICP-MS as the method of choice, in part due to the low LOD values achievable. However, in general the use of GC / HPLC-AFS provides lower LOD values than those obtainable via HPLC-ICP-MS. The paper is worth reading for those new to the field but the above mentioned caveats regarding the need for derivatisation and obtainable LOD values need to be considered as well.

The mobility, toxicity and bioaccumulation of Hg depends on its chemical form and so *compound-specific Hg isotope ratio measurements*, from which δHg values can be calculated, can help elucidate these processes. As such, the chromatographic peak width, integration time, number of acquisition points and data treatment strategy for measurements by GC-MC-ICP-MC have been systematically investigated with respect to both accuracy and precision of these measurements.[129] The investigation was carried out using the primary standard for δHg, NIST SRM-3133 and a secondary standard, NIST RM-8610 and Hg species were ethylated before injection onto the GC column. The GC eluent was combined, via a heated metallic block, with the aerosol of a conventionally nebulised Tl solution which was used for mass bias correction purposes. As GC can give narrow peaks, in the order of 2 - 5 seconds in duration, which leads to a limited number of data points per peak due to the need for MC instruments to have longer dwell times than quadrupole instruments, the temperature conditions were varied to provide peak widths of up to around 50 s to allow a greater number of data points per peak. A further evaluation was made of detector response time lag, which is different for each detector in an MC array, which is reported to be the main cause of drift in the measured isotope ratios when separation techniques are coupled with MC-ICP-MS. Three different approaches were evaluated for the calculation of isotope amount ratios, peak area integration, linear regression slope (LRS) and calculation on a point by point basis for each integration time of the chromatographic peak. The aim was to be able to acquire accurate and precise δHg data when using narrow GC peaks, thus saving time particularly for samples which contain many Hg species. Using the developed data treatment protocols, by which isotope ratios were calculated by the LRS approach, and corrected for time lag and mass bias effects, good agreement between the found and certified δHg values for both the CRM and RM studied, was achieved, e.g. for NIST RM 8610 the found δ^{201}Hg values was -0.27 ± 0.35‰ and the certified value is -0.27 ± 0.02‰. Typical precision was 0.4‰ and the accuracy and precision in this work is comparable to that obtained with other work where broad GC peak widths are used for the measurements. The paper contains a wealth of detail, with more available as supplementary information, and is recommended reading for practitioners in the field.

There are a number of reports on new methods for Hg speciation to consider this year, although new can be seen as incremental changes to existing methods rather than a completely new approach. In one of these reports post column CV and photochemical vapour (PV) generation methods coupled with AFS detection were compared with respect to accuracy, precision and sensitivity[130]. Mercury species were extracted from five aquatic tissue and two hair CRMs with 10 mmol L^{-1} APDC in 80% MeOH for 30 mins at 60 °C in a hotblock followed by 15 minutes UAE. Separations were carried out in 10 minutes on a C18 column (250 x 4.6 mm, 5 µm) with a mobile phase of 80% (v/v) MeOH and 1.5 mmol L^{-1} APDC flowing at 1 mL min^{-1}. The CV method used 0.05

mol L^{-1} bromide/bromate in 1.2 mol L^{-1} HCl and UV as an oxidant followed by reduction of the generated Hg^{2+} with 2% (m/v) $SnCl_2$ dihydrate in 1.2 mol L^{-1} HCl to generate Hg^0. The PV method used only one reagent, 25% (v/v) acetic acid and UV followed by a make-up flow of water to generate Hg^0. In both cases Ar was sued as the carrier gas to transport the Hg^0 to the AFS detector. The results presented show similar MeHg recoveries for each vapour generation method (93 – 104% and 93 – 105% for CV and PV respectively) and precision (0.3 – 5% and 1.1 – 5% for CV and PV respectively) but with a lower LOD value for the PV method (30 and 78 ng L^{-1} for PV and CV respectively). The method was then applied to tuna fish samples in which the MeHg content varied from 1.1 – 6.6 mg kg^{-1} and these results were found to be in good agreement (linear regression, slope 1.0077, R^2 0.9824) with the values obtained when the sample extracts were analysed using a GC-pyrolysis-AFS method. A second group of workers also report the use of UV assisted PV for Hg speciation this time interfacing the device between an HPLC column and two separate AAS detectors, one being a conventional line source instrument and the second being a continuum source instrument.[131] Mercury species were extracted from two fish tissue CRMs (ERM-CE464 tuna and NRCC DOLT-4 dogfish liver) using a variety of different reagents and heating techniques previously reported in the literature. Extraction using 6.25% v/v TMAH and 0.05 mol L^{-1} with heating and reflux at 75 °C for 30 minutes was chosen for further work due to its compatibility with the HPLC-UV-PG-AAS method used as the other methods evaluated caused either signal suppression or Hg species transformations. After optimisation of the HPLC and PV conditions, which are discussed fully, four Hg species (iHg, MeHg, EtHg and PhHg in elution order) were separated in 16 minutes with good baseline resolution on a C18 column (250 x 3 mm, 3 µm) with a mobile phase of 40% v/v EtOH, 0.1% v/v 2-mercaptoethanol content and a CH_3COOH/CH_3COONa buffer (20/20 mmol L^{-1}) at pH 4.75 flowing at 0.3 mL min^{-1}. The LOD values ranged from 0.47 µg L^{-1} for iHg to 2 µg L^{-1} for PhHg and recoveries from the CRMs analysed ranged between 72 – 93%, suggesting that the extraction method needs further improvement. A QuEChERS-like method combined with an automatic sample pre-treatment apparatus has been developed the determination of Hg species in aquatic animal samples.[132] The extraction device consists of two concentric tubes and a series of microporous membranes for liquid transfer and gas pressure balancing. The procedure involves sample loading, shaking extraction, centrifugation, shaking clean-up and centrifugation for phase separation and the device design and optimisation of use is described in detail in the paper. The devices allowed Hg species to be quantitatively extracted from 12 samples in 25 minutes which is a good sample throughput compared with current methods in the literature. The figures of merit for the entire procedure, which included HPLC-AFS separation and detection of the extracted Hg species, are compared with 10 other data sets from the literature and the LOD values obtained, between 1.2 and 2.4 ng g^{-1} depending on Hg species are similar to those presented for other GC and HPLC AFS methods. Recoveries of MeHg from a fish tissue CRM (GBW 10029) was 102% and spike recoveries of iHg and EtHg ranged between 90 and 101%.

Four different research groups report on *the use of functionalised magnetic particles for the extraction of Hg species from aqueous samples*. The first of these papers covers the preparation of Fe3O4@SiO2@ glycidyl methacrylate ((GMA)-S-SH) NPs.[133] The NPs were used to extract iHg, MeHg and PhHg from spiked water samples, adjusted to a pH of 4, in 5 minutes followed by NP collection, elution of the retained HG species with 0.1 mol L^{-1} HNO_3 and 4% thiourea giving enrichment factors of > 300 for the Hg species which were separated and quantified by HPLC-ICP-MS. The adsorption capacity of the NPs was found to be 141, 142 and 32.1 mg g^{-1} for iHg, MeHg and PhHg, respectively, which compared favourably with the literature data presented for other magnetic NPs used for this purpose. The LOD values obtained were 0.40, 0.49 and 1.4 ng L^{-1} with RSD% values of 8.5% or better (n = 5). The found mass fractions of Hg species, after extraction with 6 mol L-1 HCL and UAE for 20 minutes, in four CRMs, GBW 10043 rice, GBW 07 425 and 07427 soils and DORM-2 dogfish muscle, were in statistical agreement with the certified values ($t_{0.05, 2}$= 4.30). The method was subsequently applied to spiked farmland water, soil and rice samples and the recoveries ranged between 84 and 111%. The second paper covered here describes the preparation of two Zwitterion functionalised NPs, each type having an anionic sulfonic group and either a N^+ or S^+ cationic terminus, and used packed into a minicolumn.[134] The minicolumn was fitted into a six port injection valve and 120 mL of water sample (river, lake or seawater which had been treated by passage over a Ag based column to remove Cl^- ions) pumped through in 15 minutes, washed for 1 minute with water and then eluted onto an HPLC column with 60 mmol L^{-1} ammonium acetate and 0.15% L-cysteine at pH 8 in 4% MeOH at a flow rate of 1 mL min−1. Detection of the eluted Hg species was by ICP-MS. Enrichment factors were 100 for Hg species (iHg, MeHg and EtHg) concentrations of up to 500 ng L^{-1}, LOD values were all < 0.8 ng L^{-1} and the column was useable for 65 extractions before SEM analysis showed the physical structure beginning to degrade. The third paper reviewed here describes the modification of Fe_3O_4 NPs with nanaocellulose for Hg species extraction from water samples.[135] After optimisation of the extraction parameters, pH (3 adjusted with ethanaoic acid), amount of nanomaterial (10 mg), time and volume of sample (10 mL), *in situ* ethylation with $NaBEt_4$, extraction into hexane and analysis by GC-pyrolysis-AFS LOD values of 5.6 and 4.0 pg mL^{-1}, for iHg and MeHg respectively were obtained. The enrichment factor for both species was 300 and spike recoveries into real water samples at two levels, 0.1 and 1.0 μg L^{-1}, ranged from 81 to 98%. Finally, the adsorbtion of dithizone onto the surface of magnetite-reduced graphene oxide composites has been reported and the material used to extract Hg species from environmental samples.[136] After method optimisation enrichment factors of 400 and 380 (from a 400 mL water sample) were obtained for iHg and MeHg, respectively. The LOD values were 0.48 and 0.17 ng L^{-1} for iHg and MeHg, respectively. Spike recoveries were 92-110% and RSD% values were < 3.1% in real water samples. The HPLC-ICP-MS method was then applied for the determination of Hg species in river water, ground-water, sea water and sewage effluent samples.

Two papers from the same research group report on the *detection of Hg species in produced waters* (PW) this year. The first paper describes a laboratory based procedure whereby Hg species were extracted at 40 °C using a surfactant, 0.5% w/v Triton X-114 at pH 3, and UAE for 40 minutes followed by distillation at 130 °C, propylation and separation and detection using GC-pyrolysis-AFS.[137] The LOD values obtained were 5.0, 8.0 and 11.0 pg L^{-1} for Hg^{2+}, MeHg and EtHg, respectively. Recoveries of 92% for Hg^{2+}, 87% for MeHg and 86% for EtHg were obtained, when compared to an aqueous standard solution containing the same analyte concentrations and the method was suitable for samples with an oil content between 20 to 60 mg L^{-1}. For four PW samples the iHg and MeHg contents ranged between 0.3 – 0.6 and 0.2 – 0.5 μg L^{-1}, respectively. The second paper aimed to develop a method for HG 'speciation' in PW that could be used offshore.[138] In this method the PW samples were centrifuged and the supernatant diluted to give salt and oil concentrations in the ranges of 2 to 6 g L^{-1} and 2 to 6 mg L^{-1}, respectively. Subsequently, the iHg content was determined by CV-AAS using $SnCl_2$ as the reductant and the total Hg content was measured in a similar manner after exposure of the sample UV irradiation (253 nm with no power level or duration given) to oxidise organoHg species to iHg and the organoHg content calculated by difference, so the method is really fractionation rather than speciation. The method LOQ is reported as 12 ng L^{-1}. For 4 PW samples the iHg and organoHg contents ranged between 0.2 – 0.8 and 0.02 – 0.07 μg L^{-1}, respectively. In both papers the authors also mention recoveries from fortification experiments, at three different levels, and all of these 'recoveries' are 100% which suggests that they are actually standard addition calibrations with a very high degree of linearity for the calibration curve.

The determination of *Hg species in biota* has also been reported on this year. One of these describes the measurement of Hg species in Chinese Rare Minnow after exposure to EtHg at 10 different concentration levels ranging from 0 – 80 μg L^{-1} as Hg[139]. Mercury species were extracted from homogenised tissues with 25% m/v KOH in MeOH, shaking, extraction of organoHg species into CH_2Cl_2 and then back extraction into 10 mmol L^{-1} Na thiosulfate. The Hg species were separated and detected in 35 minutes, using HPLC-AFS the details of which are given in a cited reference, and from the chromatogram presented does not look suitable for iHg quantification as this species appears to be very poorly eluted from the column. The method was validated using DORM-2 CRM (no results given) and by spiking of fish muscle with EtHg for which a recovery of 85% was obtained. For some fish extracts an unknown Hg species was detected which was later identified as diethylmercury by retention time matching with an in-house synthesised standard. The quantification of Hg species in blood from newcomer women to Toronto, who originate from SE Asia, has been undertaken using ID-SPME-GC-ICP-MS.[140] Aliquots of whole blood were spiked with the ^{199}Hg and ^{201}Hg enriched MeHg and iHg respectively, TMAH, vortexed and digested for 20 h at 90 °C followed by SPME (PDMS/DVB coated fibre) and derivatised with $NaBPr_4$ (1% w/v). Chromatographic separation of Hg species was achieved using a ZB-5 column (30m × 0.25 mm; film thickness: 0.25 µm) and the eluent was transferred to a quadrupole ICP-MS instrument *via* a transfer line heated to 260 °C. Noncertified RMs from the PCI Interlaboratory Comparison Program for Metals in Biological Matrices (PC-B-M-1201 and PC-B-M-1203) were used for quality control purposes. Blood MeHg concentrations ranged from 0 to 25 µg L^{-1} with the geometric mean value being 2.66 µg L^{-1} which was higher than that reported in a study of native Canadian women of 0.57 µg L^{-1}.

Two papers report on the simultaneous separation of As and Hg compounds. In the first of these a C18 column (5 µm, 4.6 × 50 mm) with a gradient elution (mobile phase of A: 4 mmol L^{-1} TBAH (pH 6.0) and B: 4 mmol L^{-1} TBAH + 20 mmol L^{-1} Cys (pH 6.0)) flowing at 1 ml min $^{-1}$ separated As^{III}, As^{V}, DMA, MMA, iHg, MeHg and EtHg in 11 minutes with good baseline separation for all species although it does look as if the As^{III} might elute with the solvent front. Species detection was by ICP-MS. Method optimisation is discussed in detail and validation was by analysis of two CRMs, ERM-CE464 tuna (MeHg and total Hg) and NMIJ 7532-a rice flour (As^{III}, As^{V}, DMA) and all of the found values were stated to be in agreement with the certified values. The linear range was 0.5 – 500 µg L^{-1} for the As species and 0.1 – 100 µg L^{-1} for the Hg species. Detection limits were < 0.2 µg L^{-1} for As species and < 0.35 µg L^{-1} for Hg species. The method was applied to extracts (cited method) of lotus root and the major species were found to be DMA (3 – 8 µg kg^{-1}) and MeHg (0.6 – 1.0 µg kg^{-1}). The second study uses a previously published method to assess the As an Hg species content of shellfish from coastal cities in China.[60]. The method uses a C18 column (5 µm, 4.6 × 150 mm) a gradient elution (mobile phase of a: 5 mmol L^{-1} TBAH and 10 mmol L^{-1} $NH_4H_2PO_4$ and B: 5% (v/v) MeOH, 0.1% (m/v) L-cysteine and 0.06 mmol L^{-1} CH_3COONH_4) to separate As^{III}, As^{V}, DMA, MMA, iHg, MeHg and EtHg in 10 minutes with ICP-MS detection. This HPCL method does not baseline resolve As^{III}, DMA and MMA and there is also what is described as a ghost peak in the Hg chromatogram for both standards and samples. This appears after the MeHg signal but before that of EtHg and iHg. No discussion of this ghost peak is contained within this report but it was discussed in the original report on the method. Three different extraction procedures, MAE with HNO_3, UAE with HCl and UAE with $NH_4H_2PO_4$ and *L*-cysteine, were evaluated for the extraction of As and HG species from shellfish tissue. Of these, MAE with 1% HNO_3 gave the larger recoveries, 85 and 65% for As and Hg, respectively and so was chosen for use. Method LOD values for each As or Hg species were all less than 0.1 mg kg^{-1}. Of the seven shellfish species analysed *Ruditapes philippinarum*, a saltwater clam, had the highest As and Hg content mainly as DMA and MeHg. From the data presented for the shellfish species it would appear that the sum of the species (both for As and Hg) was significantly less, about half, than that of the total elemental content determined after an MAE procedure with HNO_3 and H_2O_2 suggesting that there were either other As and Hg species present in the samples that were not detected or that the extraction efficiencies for real samples were poorer than those obtained for the CRM used. Table 2 shows examples of other applications of Hg speciation presented in the literature during the time period covered by this ASU.

Table 2 shows examples of other applications of Hg speciation presented in the literature during the time period covered by this ASU.

Table 2 Applications of Speciation Analysis: Hg

Analyte species	Technique	Matrix	Sample treatment	Separation	LOD	Validation	Reference
Total Hg, organo Hg	ET-AAS	Sediments, salt marsh plants, invertebrae fish muscle and liver	Total Hg: Direct analysis of solids. organoHg: 18% KBr, 5% $CuSO_4$ in 5% H_2SO4, extraction into toluene, back extraction into $Na_2S_2O_3$	Selective extraction.	Not given	ERM-CD200 (seaweed), ERM-CE278k (mussel tissue), Tort-3 (lobster hepatopancreas) Dorm-4 85 - 105% recovery	141
Total Hg, organo Hg	ET-AAS	Sediments,	Total Hg: Direct analysis of solids. organoHg: 18% KBr in 5% H_2SO4, extraction into DCM, back extraction into water, ehtylation	Selective extraction.	Not given	PACS-3 marine sediment, NIST 2976 (mussel tissue) 89 and 99% recovery, respectively	142
iHg,	HPLC-	Hair	MeHg: 10% TMAH, 80	C18 column, 0.5	0.005 mg	NIES CRM No 13	143

EtHg, MeHg	ICP-MS		°C 2 hr	g L^{-1} L-cysteine, 1% MeOH. 10 minutes	Hg kg^{-1} for all species	(human hair) MeHg 102% recovery	
organo Hg	ET-AAS	Sardine	MAE, 1000 W, 110 °C, 10 min, 30% HCl and toluene	Selective Extraction	2.2 μg kg^{-1}	Tort-2 (lobster hepatopancreas) 106% recovery	144
MeHg	GC- CV-AFS	Rat faeces	1 mol L^{-1} $CuSO_4$, 25% $HN)_3$, extraction into DCM, back extraction into H_2O	GC conditions not given	Not given	DORM-2 (dogfish muscle) 90 -110% recoveries	145
iHg, EtHg, MeHg	ID-GC-ICP-MS	Blood, hair, urine	MAE, 25% TMAH, propylation	Not given, cited reference	Not given	NIST 955c (Caprine Blood), IAEA-086 (Human hair), spiked urine. > 88% recoveries	146
Hg^0, $HgCl_2$	ET-AAS	Flue gases	None needed	Differential volatilisation of Hg (Hg^0 150 °C, $HgCl_2$ 900 °C)	Not given	None given	147
iHg, organo Hg	ET-AAS	Waters, fish tissue extracts	Hollow fibre LPME. OrganoHg: toluene, $Na_2S_2O_3$, iHg: diethyldithiocarbamate, 1-octanol	Selective extraction	iHg 0.143 ng mL^{-1}. organoHg: 0.063 ng mL^{-1}	NIST 15666b (oyster tissue), organoHg 97% recovery	148
iHg, organo Hg	ET-AAS	Waters, fish tissue extracts	iHg; dithizone, choline-phenol based DES, organoHg: Choline-phenol based deep eutectic solvent	Selective extraction	iHg 0.073 ng mL^{-1}. organoHg: 0.091 ng mL^{-1}	NIST 15666b (oyster tissue), organoHg 107% recovery	149
iHg, organo Hg	Hg-AFS	Waters	Sequential cloud point extraction, KI and methyl green (iHg) then APDC (organoHg)	Selective extraction	iHg 0.007 ng mL^{-1}. organoHg: 0.018 ng mL^{-1}	Spike recoveries, 95% or greater.	150

3.14 Nickel

An interesting approach focused on *Ni speciation* is the work developed by Wang *et al.*[151] related to the transformation of Ni compounds contained on an electroless Ni plating effluent along a sequential waste treatment scheme. For this purpose, an HPLC-ICP-MS methodology was developed consisting of the application of different separation conditions: SEC (125 A, 7.8 x 300 mm, 3.5 µm column and 97% MeOH and 20 mmol L^{-1} NH_4AC as the eluent at a flow-rate of 1.0 mL min^{-1}), AEC (4 x 250 mm column and 0.02 mol L^{-1} NaOH as the eluent at a flow-rate of 1.0 mL min^{-1}) and CEC (4 x 250 mm column and 0.02 mol L^{-1} methanesulfonic acid as the eluent at a flow-rate of 1.0 mL min^{-1}). Effluent samples were taken at four representative sites: the outfalls of physicochemical treatment, biochemical treatment, Fenton treatment and biological aerated filter. The results demonstrated the presence of Ni –complexes similar to EDTA-Ni as the dominant Ni species in the effluents. Analysis by HPLC-ES-MS/MS, using the same eluent conditions employed for the SEC separation were applied to accurately determine the molecular weight of the Ni-compounds in the effluents. Several Ni-complexes containing heterocyclic stereo structures were detected. According to the indexes of H deficiency, both Fenton and Biological aerated filter treatments provided the highest efficacy in degrading Ni-complexes. This finding was corroborated by a decrease in Ni concentration along the sequential treatment scheme with the biggest decline after the Fenton unit, thus showing the strong ability of hydroxyl radicals to break Ni-complexes. However, the authors point out the need to search for alternative technologies to mitigate the presence of refractory residual Ni compounds in wastewater samples.

3.15 Platinum

An interesting selection of papers from different research groups have been published on *the development of methods to measure platinum anti-cancer drugs* using a diverse range of novel approaches including: monolithic conjoint liquid chromatography (CIC) coupled to ICP-MS with IDA calibration for carboplatin-protein adduct measurement; CE-ICP-MS for oxaliplatin enantiomer characterisation; and LA-ICP-MS for cisplatin-albumin adduct determination.

The *carboplatin protein adduct* work initially investigated two separation methods for their determination in spiked human blood: asymmetrical flow field-flow fractionation (AF4); and monolithic CIC.[152] The AF4 method was unable to provide baseline separation for the three protein adducts formed with albumin, transferrin and IgG, but was useful in providing information on the formation of the different adduct isoforms during optimisation of the conditions for calibrant and isotopic spike production. The monolithic CIC approach proved more successful and also more flexible, because it was possible to investigate the use of different chromatographic stationary phases by interchanging the monolithic disks. Initially the method used a single affinity based disk, containing protein G for the retention of IgG, followed by two weak AE disks containing diethylamino groups (DEAE), placed together into the same housing of the CIC device. This was coupled in series to UV-Vis and ICP-MS detectors and revealed that Pt-HSA adducts represented more than 96% of the protein-bound Pt in the serum, whereas Pt-Tf and Pt-IgG represented only about 2% each. Subsequently the protein G disk was removed and the separation of transferrin and albumin optimised using a binding buffer A of Tris HCl (50 mmol L^{-1}) and $NaHCO_3$ (30 mmol L^{-1}) at pH 7.4 and a gradient elution buffer of A plus NH_4Cl (2 mol L^{-1}) at pH 7.4. Calibration used ssIDMS with a spike comprised of a carboplatin-HSA adduct containing an enriched ^{194}Pt isotope. The LOD values achieved for carboplatin–HSA adducts with the IDA method were 0.005 and 0.003 ng g^{-1} for ^{194}Pt and ^{195}Pt, respectively. These values are about two orders of magnitude lower than those previously reported in the literature. In the absence of a suitable CRM for method validation, the method accuracy was assessed by evaluation of recovery experiments on the ERM-DA470k/IFCC serum incubated with carboplatin and spiked with the natural isotopic carboplatin–HSA calibrant. The average recovery was 102 ± 2.5%. It will be interesting to see whether the method is used in real patient samples or in the production of CRMs.

A method for the *baseline separation of oxaliplatin enantiomers in pharmaceutical and urine samples at atto-molar concentration levels* used CE-ICP-MS with an "in-house" designed interface.[153] The two biggest drawbacks of coupling CE to ICP-MS are the very low sample volumes possible, as coupling to the detector is not straight-forward, as electrical conductivity throughout the system is required to maintain the separation, and achieving useable LOD values difficult. The developed CE-ICP-MS interface consisted of a cross-piece, a grounding electrode, and an inlet for introduction of a sheath liquid (placed in the inlets of the cross-piece), and a self-aspirating MicroMist concentric nebuliser with a flow rate of 107 ± 6 µL min^{-1}. The sheath liquid (a 20-times-diluted buffer solution without a chiral selector) was introduced by self-aspiration to the nebuliser inlet, where it was mixed with an effluent from the CE capillary. Separations were performed in fused silica capillaries of 64.5 cm length x 25 µm ID with a borate buffer (40 mmol L^{-1}) containing sulfated β-cyclodextrin (60 g L^{-1}) a chiral selector additive, which interacted with the oxaliplatin enantiomers to form negatively charged diastereomeric complexes, both of which migrated in the opposite direction to the electro-osmotic flow. The developed method provided a wide dynamic range (0.1 – 500 mg L^{-1}) and detection of approximately 49 fg, or 125 attomol, of oxaliplatin enantiomers in the CE injected sample (0.8 nL), with an LOD and LOQ of 64 and 116 µg L^{-1} of oxaliplatin, respectively. The CE-ICP-MS method could be used for pharmacokinetic studies dealing with the chiral metabolism of oxaliplatin.

The *use of LA to couple planar SDS PAGE to ICP-MS detection* has been reported for the measurement of cisplatin-HSA adducts from drug:HSA samples incubated at different ratios.[154] Whilst some of the earliest LA-ICP-MS work involved the analysis of cisplatin in GE separations this paper describes a useful calibration approach involving doping the gel with known Pt concentrations to prepare standards. Unfortunately, addition of cisplatin or platinum standard stock solutions to the polyacrylamide gel matrix was not successful, as both yielded poor linearity, thought to be related to the poor retention of small molecules in the matrix used. Instead HSA–cisplatin adducts were used as standards, without running an electrophoretic separation, the standard gels were dried and analysed by LA–ICP-MS. Using this method, standards containing 0, 0.05, 0.1, 0.25, 0.5, 1, 1.5, and 3 mg L^{-1} Pt were prepared. To correct for a significant drift of the signals over time, inhomogeneity in the gels and varying ablation efficiency, the ^{29}Si and ^{13}C signals were also monitored. The source for m/z 29 was thought to be a combination of signals from the Si-containing glass backing plate and two potential polyatomic interferences ($^{13}C^{16}O^{+}$ and $^{15}N^{14}N^{+}$). The latter provided a back-ground level for m/z 29, even if the laser was switched off between analysing protein bands, and provided an indication of the constant ablation performance of the laser. The signal for ^{13}C was used to correct for variations in the gel matrix. Both m/z 13 and 29 were applied to correct the ^{195}Pt signals and this resulted in excellent repeatability and standard curves with high linearity and a LOD for ^{195}Pt of 18 µg L^{-1}, while the LOQ was 37 µg L^{-1}.

3.16 Phosphorus

An interesting paper has appeared this year on the use of *HPLC-ICPMS/MS to determine six phosphorous compounds of environmental interest*, aminomethylphosphonic acid (AMPA), ethephon, fosamine, glufosinate, glyphosate and phosphate in waters.[155] Separations were performed on an AE column (Hamilton PRP-X-100, 250mm x 2.1 mm, 5µm) column with 2.0 mmol L^{-1} malonic acid at pH 5.3 as the mobile phase flowing at 0.6 mL min^{-1} for 14 minutes. The effect of different experimental conditions such as mobile phase composition and cell gas modes on phosphorous separation and detection, respectively are described in detail. The use of oxygen as a reaction gas and ICP-MS/MS detection provided an average 20-fold improvement in the LOD (0.1-0.3 µg P L^{-1}) in comparison to the method based on single quadrupole ICP-MS detection. In absence of a RM, method accuracy was determined by performing recovery studies in non-enriched water samples spiked with four levels of concentration of mixed standard solutions. The recovery of all analytes was in the range of 86-112%, respectively with RSD values lower than 10%. Only phosphate was detected, 2.7 ± 0.3, 2.0 ± 0.15 and 14.0 ± 0.3 µg L^{-1} in ground, tap and river water, respectively in the unspiked samples.

3.17 Selenium

The *development of Se functional food and beverages has been reviewed* this year by Adadi *et al.*[156] The review describes some of the technologies employed for designing Se-enriched functional beverages (such as Se-enriched beer, selenised kwas) and nutritional supplements (selenised-yeast, selenised-algae and selenised-flour). Of special relevance for the readers is the description of the mechanisms involved in the transformation and bioaccumulation of Se in different Se-enriched products as for instance algae. Finally, the impact of Se on gut microbiota, its biovailability as well as the factors that need to be considered when designing functional food are described. Interestingly, the future role of nutrigenomics in designing functional food is highlighted. The review includes tables with detailed information on the Se-functional food and beverages appeared in the literature. More than 150 references are included although only a few are concerned with the detection of Se species by atomic spectrometry.

Most of the papers that have appeared this year on *Se* speciation deal with its essentiality. *The biological and health relevance of selenoneine* (a Se-analogue of ergothioneine) has generated increasing interest in Se research. Selenoneine has been postulated to have a key role in protecting against MeHg induced toxicity and oxidative stress. Selenoneine was isolated originally from the blood of blue tuna and it has been also found as the major Se-specie in blood cells of fish-eating populations. Two papers report on selenoneine quantification in biological samples using LC-ICP-MS/MS. In the first study the method was applied to determine selenoneine and its methylated metabolite in red blood cells of 210 individuals belonging to the Artic indigenous population of Inuit of Nuvalik (Canada).[157] This population exhibits one the highest Se status in the world because of its high consumption of marine mammal based foods such as beluga, walrus, seal and fish eggs. In this sense, the authors of the study also investigate the presence of selenoneine in Beluga mattaaq, a delicacy consisting of the skin with the attached fat layer, which is a major source of dietary Se for Inuit of Nuvalik. Total Se quantification in blood samples and beluga skin was performed by ID-ICP-MS by using the ^{78}Se:^{77}Se isotope ratio. Selenoneine and its methylated metabolite were isolated from blood samples by treating them with 50 mmol L^{-1} dithiothreitol in aqueous solution followed by a filtration of the homogenate through a 10 kDa cut-off centrifugal filter. Selenium species separation in the extracts was achieved by using an Atlantis T3 reversed-phase column coupled to the ICP-MS/MS with a mobile phase consisting of 0.5 mmol L^{-1} tetrabutylammonium phosphate, 0.5% heptafluorobutyric acid and 2% MeOH (pH 2.3). Under these chromatographic conditions, selenoneine and methylselenoneine were baseline separated in less than 4 minutes. The identification of the Se-containing peaks was by retention time matching. In addition, HPLC-QTOF-MS measurements were performed with the aim of confirming the identity of Se-compounds. For this purpose, a ZIC-HILIC column was employed along with a mobile phase consisting of ACN and 10 mmol L^{-1} $(NH_4)_2CO_3$ at a flow rate of 250 µL min^{-1}. One of the most relevant aspects of the study was the synthesis and characterisation of selenoneine and methylselenoneine as they are not commercially available Selenoneine was biosynthesised using the genetically modified strain of *Schizosaccharomyces pombe*. This yeast when growing in a S or Se-enriched medium is able to produce ergothioneine and selenoneine, respectively. Selenoneine from the lysates of *S.pombe* was further isolated by cation-exchange-SPE and its identity confirmed using UHPLC-QTOF-MS. Methylselenoneine was prepared through the derivatisation of selenoneine with diazomethane. The results obtained revealed that Se was the main Se-specie in red blood cells with a median Se concentration of 413 μg L^{-1} (range = 32.0-3230 μg L^{-1}), representing 54% of the total Se present. Quantification of selenoneine in Beluga mattaaq samples provided a median concentration of 1.8 μg Se g^{-1}. Selenomethylselenoneine was detected in red blood cells but not in Beluga mattaaq, suggesting selenoneine methylation in humans. The authors attribute the presence of selenonine in Beluga whale to the composition of its skin microbiome able to biosynthesise selenoneine. The second work deals with the simultaneous determination of selenoneine and ergothioneine in human blood cells.[158] Several sample treatments are described in detail. The best results, in terms of selenoneine and ergothioneine stability and extraction efficiency were achieved by applying a method consisting of the lysis of blood cells with cold water followed by protein removal by cut-off filtration (3000 Da). For the separation and detection of ergothioneine and selenoneine in the resulting lysates of blood cells a reversed-phase (Atlantis dC18, 4.6 x 150 mm) column coupled to ICP-MS/MS was employed by using a mobile phase composed of 20 mmol L^{-1} of ammonium formate (pH 3.0) and 3% MeOH at a flow-rate of 1.0 mL min^{-1}. The addition to the mobile phase of the reducing agent TRIS (2-carboxyethylphoshine), (TCEP) was evaluated. One of the problems found in selenoneine analysis is its autoxidation resulting in the formation of a dimer with a different chromatographic behaviour compared to the oxidised form. Moreover, both oxidised and reduced forms can be simultaneously present in samples and standard, therefore hindering the straightforward determination of selenoneine. The addition of TECP to the mobile phase allowed the authors to transform the selenoneine to a single reduced form while the rest of S and Se compounds were not affected by the presence of the reducing agent. The validity of the method was evaluated by spiking blood cells with aqueous solutions of commercially available ergothioneine and selenoneine isolated from *S.pombe*. Under optimal conditions an average recovery of 80 - 85% was obtained for spiked human blood cells samples. The LOD values were determined to be 0.10 mg S L^{-1} for ergothioneine and 0.25 μg Se L^{-1} for selenoneine. The precision (RSD) was 10 - 1% for ergothioneine and 25 - 2% for selenoneine. The method was applied to blood cell samples from 3 volunteers which provided selenoneine and ergothioneine concentrations in the range of 3.25 to 7.35 μg Se L^{-1} and 0.86 and 6.44 mg S L^{-1}, respectively. Selenoneine concentration in the lysates of blood cells was not affect by the presence of TECP, and only a slightly better resolution in the separation of MeSeCys from selenoneine was achieved. A study has been undertaken to investigate the effect of different levels of dietary Se^{IV} and L-SeMet, and Se, SelP, SeAlb and SeMet content in plasma, colostrum and milk of 32 sows.[159] For this purpose, sows were submitted to 4 treatments from a 30 day prepartum throughout on average of a 32 day lactation period. Total Se concentration was determined by ICP-MS after specific sample treatment depending on the nature of the sample. Feed samples were digested with HNO_3 and MAE whereas colostrum and milk samples were homogenised at 37 °C centrifugation and ultrafiltration. Before analysis, plasma, colostrum and milk samples were diluted with a mixture of butanol, EDTA and Triton X-100. The concentration of the Se species in the samples was measured by HPLC-ICP-MS. The SeMet in the resulting < 3 KDa filtrate were separated on a C18 column (C18, 3 x 75 mm, 2.7 µm) column using 5:95 MeOH:H_2O with 0.1% HFBA as the mobile phase. The SelP and SeAlb concentrations were evaluated by affinity chromatography by employing two chromatographic columns (HiTRAP-Heparine and HiTRAP Blue) coupled to the ICP-MS with 0.05 mol L^{-1} and 1.5 mol L^{-1} NH_4Ac as mobile phases in a gradient elution mode. Selenium species were identified by retention time matching with commercial standards. Only peaks assigned to known Se-species were considered for quantification. The SeMet calibration curves were employed to quantify the three Se-species (SelP, SeAlb and SeMet) which may have led to inaccuracies due to any compound specific responses. A noticeable increase in the level of total Se, SelP and SeAlb and SeMet in colostrum was observed for those animals supplemented in Se-Met enriched diet compared with sows fed with Se^{IV}. According to the data obtained, authors recommend the substitution of dietary selenite with SeMet in sows to decrease oxidative stress and to improve their average daily weight gain.

The *role of Se on As accumulation* is gaining in interest in environment studies.[160] The application of Synchrotron-XRF imaging enabled the location of As and Se in the brain of fishes co-treated with As^{III} and SeMet. Mapping using XRF revealed an increase of As accumulation in brain and in other tissues in presence of SeMet along with a reduction in Se accumulation. This antithetical effect of As and Se was attributed to the interference of SeMet and its methylated metabolites with the methylation of As, thus reducing the depuration rate of As from the body. The developed study is quite novel and open new insights in the evaluation of this type of interaction.

3.18 Silver

Two papers report on *Ag speciation studies* this year. Elemental speciation is now being applied to Ag nanoparticles and this dissolution products and this topic has been reviewed[161]. The review, which contains 115 references, covers extraction procedures (mainly CPE and SPE), applications involving CE, FFF, HPLC all coupled with ICP-MS as the detector and also includes a brief section on sNP ICP-MS. Most of the data presented is in a tabular format which includes the analyte(s), sample type and LOD values achieved. The authors conclude that more accurate methods still need to be developed although on what basis this conclusion is drawn is not apparent as no method validation data is given for any of the articles cited. A method has been developed for quantifying Ag NPs and Ag+ in E. *coli*. Lysis to remove the cell wall allowed the cell surface-adsorbed Ag species to be separated from intracellular Ag species, which were extracted using TMAH and subsequently separated and quantified by SEC-ICP-MS. The SEC column is described as an 'amino column with a pore size of 1000 Å' and the mobile phase was 2% (v/v) FL-70 (a surfactant) and 2 mmol L^{-1} $Na_2S_2O_3$ flowing at 0.5 mL min^{-1} with a run time of 8 minutes per sample. Using this system baseline separation was achieved between Ag NPs and Ag^+ complexes but not between Ag NPs of different sizes (10, 20 and 30 nm in diameter) which showed a degree of peak overlap. The LOD value was 3 ng/107 CFU/mL (where CFU is colony-forming unit) for Ag species detected. The cell wall-adsorbed Ag was calculated by difference between the total Ag and the intracellular Ag content. After exposure to Ag NPs the authors report that intracellular and cell wall-bound Ag accounted for 6 − 15% and 25 − 64% of the exposed dose, respectively, and that AgNPs could transform into complexed or free Ag^+.

3.19 Sulfur

There is a growing interest in *S as a target element for speciation studies*. George *et al*. review the state of the art and the latest developments in the use of X-rays from synchrotron radiation sources for S determination [162]. Sulfur has been considered by several authors as a spectroscopically silent element due to the lack of spectroscopic techniques for probing its chemistry. The review highlights the advantages of X-ray absorption spectroscopy and X-ray emission spectroscopy for S determination in a wide variety of systems ranging from pure compounds to complex mixtures and even structured systems such as biological tissues. In this line, examples of the application of synchrotron-based techniques to determine S in samples as diverse as fossil fuels, plants and fungi, mammalian cell cultures and tissues are described. Particular attention is given to a group of techniques known as advance X-ray spectroscopy that enables the determination of S distribution in tissues, thus providing information about the electronic structure and the chemistry of this metalloid in living systems. The review includes 47 references. On this theme, *Synchrotron techniques* (SR-XRF, XANES, RBS and WD-XRF) have been used in another paper for chemical speciation of S in the bark and leaf of the Amazonian plant *Andira surinamensis*.[163] Measurements by XANES revealed the presence of S in a wide variety of oxidation states. Sulfate was the main S specie in the leaves, suggesting sulfate as the main S-transporter from roots to leaves either for its storage or for its further transformation into reduced S- organic compounds.

Lately, *the use of ICP-MS/MS for S determination* has focused the attention of several researchers as it offers important advantages in terms of sensitivity and selectivity and exceptional improvement in the detection limits compared with ICP-MS. Consequently, several papers have appeared on the speciation of S by using ICP-MS/MS. Following this approach, the simultaneous determination of sulfite and other sulfur compounds in wine was carried out by using HPLC coupled to ICP-MS/MS.[164] Sulfur species were chromatographically separated by using an AE PRP-X100 (250 mm x2.1mm; 5 µm) column . Several mobile phases flowing at 0.5 mL min^{-1} were employed depending on the S compound tested: 50 mmol L^{-1} malonic acid at pH=12 (NaOH) for the separation of sulfide and sulfate; 10 mmol L^{-1} citric acid at pH=9.0 (ammonium) for the separation of thiosulfate; 5.0 mmol L^{-1} malonic acid at pH=6.5 (ammonium) for the separation of sulfur–containing aminoacids and 5.0 mmol L^{-1} malonic acid at pH 6.5 and 5% formaldehyde for the separation of free sulfite. Wine was analysed with and without applying a sample treatment step. Different sample treatments were tested: oxidation with 10% H_2O_2 with incubation at 60 °C for 1h; basic hydrolysis using 2.0 mol L^{-1} NaOH with incubation at room temperature for 1h and enzymatic hydrolysis with protease-TRIS-HCl followed by incubation at 37 °C for 6h. The main species found in red and white wine were sulfite (32 ± 3, 60 ± mg S L^{-1}) and sulfate (50 ± 3; 55 ± 1 mg S L^{-1}). The chromatographic behaviour of sulfite was highly influenced by the pH of the mobile phase and a detailed description of the chromatographic conditions affecting sulfite retention is given. A significant tailing and an important decrease in peak area was detected at neutral pH. To overcome this problem, formaldehyde was added to wine samples with the aim of forming the sulfite-formaldehyde adduct which is stable at neutral pH. Under optimal conditions a recovery of 98 - 106% was obtained for the direct determination in wine. However, the sum of detected species accounted for only 65 - 77% of total S concentration. The differences were attributed to the presence of S species strongly retained on the chromatographic columns. Mass balance was improved by applying different chemical treatments to the wine but still a difference of a 15-25% between the total S concentration and the sum of species detected were obtained. The authors highlighted the advantages of ICP-MS/MS for S determination with LOD values of 0.1 pmol L^{-1} which is 100-fold lower than the values from ICP-MS instruments equipped with a DRC. Analysis by HPLC-ICP-MS/MS was also applied to determine S and Se in *Pleurotus pulmonarius* mushroom.[165] *P. pulmonarius* was grown in a medium containing

1 mg L^{-1} of Se^{IV}. Micelium, colonised substrate and fruiting bodies at different harvesting times were analysed. Measurements by ICP-MS provided a total content of Se that varied from 31 to 26 µg g^{-1}. Aqueous extraction and enzymatic hydrolysis with Pronase were selected for releasing Se and S compounds which gave a better extraction efficiency (81%) compared to aqueous extraction. The Se and S species in the extracts were separated on a C18 column (250 mmx 4.6 mm, 5 µm) with 25 mmol L^{-1} NH_4AC, 1 mmol L^{-1} tetrabutylammonium phosphate and 2.5% MeOH at pH 5.2 as the mobile phase flowing at 1mL min^{-1}. The S and Se signals were recorded in MS/MS mode with oxygen as the reaction gas. The data obtained revealed Met and SeMet as the predominant organic species followed by significant amounts of Cys_2 and sulfate. Most of the Met (76%) and SeMet (80%) was found in the fruiting body as free form in contrast with the mycelium whilst 80% of the Met and 53% of the SeMet was incorporated into proteins. The authors attribute the presence of high amounts of free Met due to production of ethylene, as it is a key compound for this, which is essential during the fruiting process of mushrooms.

The technique of *ICP-MS/MS has been also employed as detector for GC* for both total and sulfur speciation analysis in gasolines.[166] One of the main advantages of the developed approach is the removal of the carbon based interferences from the co-elution of hydrocarbons with S-containing compounds. The application of ICP-MS/MS overcame the need to use post-column IDA, which is not straightforward with GC as this requires the on-line addition of a gaseous ^{34}S labelled standard. Total volatile S analysis was carried out by injecting the sample in a transfer line heated to 250 °C and an external calibration curve composed of a mixture of benzothiophene in hexane, with bromocyclohexane as an internal standard, and by measurement of the S/Br peak ratio. Speciation analysis was carried out by employing a HP-5 column with an oven gradient temperature from 40 to 260 °C ramping at 6 °C min^{-1} with dibenzothiophene as an internal standard. The total S concentration in commercial gasoline was found to be 6.1 ± 0.3 mg L^{-1} S. The method was validated by analysing a gasoline CRM (ERM-EF213) and the found value of 8.8 ± 0.4 mg L^{-1} S agreed with the certified value for total S of 9.1 ± 0.8 mg L^{-1} and RSD values were lower than 3%. Thiophene and monomethylthiophene isomers were found in the gasoline CRM with concentrations of 6.58 ± 0.39 and 1.31 ± 0.07 mg L^{-1}, respectively, values that were consistent with those previously reported by the authors using GC-ICP-MS and on-line isotope dilution analysis.

One paper reports on *S speciation in environmental samples using AE separation* prior to on-line isotope ratio determination by MC-ICP-MS.[167] For the separation of S species, an AE column (2 x250mm, 7.5 µm) was employed with a mobile phase consisting on 40 – 100 mmol L^{-1} NH_4NO_3 and

0.1% formaldehyde at pH 7.0. Isotope ratio determinations (^{34}S:^{32}S) by linear regression slope (LRS) and δ^{34} values were calculated. The developed data treatment protocol enabled an improvement in the accuracy and precision of isotope amount ratio measurements in the chromatographic peaks as well as overcoming blank subtraction. The application of the latter is of great interest in case of S as it is usually present at significant amounts in mobile phases and possibly ICP-MS gases. The paper includes a complete description of different approaches employed to minimise uncertainties of LRS calculations. Moreover, several methodologies to correct mass bias were investigated in detail. The developed LC-MC-ICP-MS enabled the estimation of $\delta^{34}S$ in sulfite, sulfate and thiosulfate in a single chromatographic run with RSD values of 0.3‰ for sulfite and sulfate and 0.5‰for thiosulfate. The method was also successfully used for the measurement of $\delta^{34}S$ in synthetic solutions and in environmental water samples. Overestimation of $\delta^{34}S$ measurements was only detected for sulfate in samples with high sodium:sulfate mass ratios.

3.20 Tellurium

The *environmental behaviour of Te* is still poorly understood. As such significant progress in this area requires the development of analytical techniques robust and sensitive enough to provide data at environmentally relevant concentrations. It is timely then that Fillela et al[168] have reviewed the current knowledge about the sources and environmental behaviour of Tellurium in different environment compartments (water, atmosphere, soil, sediments and biological samples). Special attention is paid to the transformation of Te once released into the environment (redox speciation, biomethylation). The last section of the review describes the analytical limitation of the most applied techniques to determine tellurium (HG-AFS, ICP-MS and voltammetry) and emphasises the need for CRMs for method validation. In addition, information about previous reviews on Te, published during the last 25 years, is included along with 70 cited references. Two papers on the application of microextraction procedures have appeared for the determination of Te in environmental samples. The speciation of CdTe quantum dots (QDs) and Te^{IV} in natural waters was performed by combining HG and head-space single drop microextraction and then quantification by GFAAS.[169] The developed protocol includes *in-situ* derivatisation of Te species for HG with 1.25% (m/v) $NaBH_4$, 1 mol L^{-1} HCl and the absence or presence of I. In the absence of I, only Te^{IV} was able to form the corresponding hydride whereas in presence of I both, Te^{IV} and CdTe QDs react with the $NaBH_4$, allowing therefore their simultaneous determination. The generated hydride was subsequently trapped onto an Au-containing microdrop exposed to the headspace of the vial where TeH_2 was generated. Once the microextraction processes is finished, the microdrop was re-extracted back into a syringe and injected in the GFAAS for determination. The factors affecting the microextraction procedure, such as the type of noble metal contained in the microdrop, microdrop volume, hydride generation conditions and I concentration are described in detail. Under optimal conditions LOD values of 0.13 µg L^{-1} and 0.03 µg L^{-1} for Te^{IV} and total Te (Te^{IV} + CdTe QDs) were attained. The accuracy of the method was validated by performing spike analysis of natural waters (superficial, ground and lake waters) obtaining recoveries in the range of 95 - 116% for Te^{IV} and 102-113% for total Te. Although the developed method constitutes a suitable and rapid tool for performing speciation of CdTe QDs and Te^{IV}, the LOD values obtained are not low enough to quantify Te at environmentally relevant concentrations. In the second paper, a dispersive solid phase extraction method for the simultaneous Se^{IV} and Te^{IV} determination by using $Fe_3O_4@SiO_2$ NPs functionalised with polyaniline and ICP-MS is presented.[170] The resulting Te^{IV}-Se^{IV} loaded NPs were separated from the solution with an external magnetic field followed by treatment with 0.1 mol L^{-1} NaOH. The in-house synthesised $Fe_3O_4@SiO_2$PANI NPs were fully characterised in terms of size distribution, morphology, composition and adsorption capacity by employing a great variety of techniques. The paper also provides a detailed description of the parameters affecting extraction efficiency: pH, type of eluent, sample volume, adsorption/settling time and the effect of co-existing ions. The method was applied to determine Se^{IV} and Te^{IV} in water samples. The LOD values for Se^{IV} and Te^{IV} were found to be 5.3 and 1.2 pg mL^{-1} with RSD values of 8 and 4%, respectively. The enrichment factor was reported to be 100. The method was validated with environmental water sample CRMs (GSB50029-94 and GBW(E) 08504) certified in total Se and Te, respectively. The method was applied to determine of Se^{IV} and Te^{IV} in river, lake and seawater water samples with recovery values within 84 - 108% range.

3.21 Thallium

The optimisation of methods based on both *anion- and cation-exchange HPLC-ICP-MS* has been reported.[171] Both separations were based on the selective formation of a Tl^{III} complex with DTPA, the charge of which is controlled by pH. In acid (pH < 2) the complex is positively charged; whereas at pH > 6 the complex is negatively charged. As Tl^{I} does not react with DTPA, the species can be separated by either AEC or CEC. Separation was effected on a Dionex cation-exchange guard column, CS12A (with 15 mmol L^{-1} HNO_3, and 3 mmol L^{-1} DPTA as the mobile phase), or on an anion-exchange column, Hamilton PRP-X100 (with 200 mmol L^{-1} ammonium acetate and 10 mmol L^{-1} DTPA at pH 4.2 as the mobile phase). For both separations, significant compound-dependent responses (at m/z 205) were observed. The injection volume was 100 µL, and the flow rate was 1.0 mL min^{-1}, for which LOD values of 3 - 6 and 9 - 12 ng L^{-1}, for AEC and CEC, respectively were obtained. The CEC method, which was simpler and faster, was applied to the analysis of a simulated water matrix of 40 mg L^{-1} Fe^{III} and 20 mg L^{-1} oxalic acid spiked to concentrations of 2 and 8 µg L^{-1} for Tl^{I} and Tl^{III}, respectively. No information about the calibration strategy was provided, but a linear range of 0.5 to 10 µg L^{-1} for each species by each method was reported. The researchers concluded that the addition of DTPA to the mobile phase significantly improved the results compared with those of previous reports. As Tl^{III} is slowly reduced to Tl^{I} even in the presence of DTPA, they recommended that the sample storage times should not exceed one week. In a study of the effectiveness of the photocatalytic decomposition of SDS (a model organic matrix component) in the presence of Fe^{III} in natural waters, the results of SEC-HPLC-ICP were used to confirm the results obtained by anodic stripping voltammetry[172]. The size exclusion column was a Superdex Peptide 10/300 GL (310 x10 mm id, 13 µm), with 100 mmol L^{-1} ammonium acetate and 5 mmol L^{-1} DTPA, at pH 6.2, as the mobile phase. The flow rate was 0.500 mL min^{-1}, and Tl was detected at m/z 203 and 205. The researchers concluded that under the proposed photolysis conditions, Tl^{I} was not oxidised in the presence of the water matrix, SDS or DTPA even when a 100-fold excess of Fe^{III} was present. In addition, the rate of reduction of a Tl^{III}-DTPA standard increased only slightly (from 2 - 3% to 4 - 6%), though this increased to 9% in a river water matrix.

A *number of techniques were applied to the characterisation of the speciation* in *a (Tl, Sb, As)-rich pyrite ore* deposit from the southern Apuan Alps (Tuscany, Italy).[173] In addition to the information obtained by LA-ICP-MS and TEM (with EDS), speciation information was obtained from X-ray absorption spectroscopy measurements on pyrite at the Tl L3-edge (12,658 eV) at the LISA beamline (BM-08) at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). The reference compounds were Tl_2O_3, protochabournéite (approximate formula $Tl_2Pb(Sb, As)_{10}S_{17}$) and Tl_2SO_4. The researchers concluded from the XANES spectra of measured samples and reference compounds that Tl in pyrite was present as Tl^{I}. The L3-edge EXAFS results showed that Tl in the pyrite samples had a first coordination shell consisting entirely of S atoms, as attempts to fit the first shell with oxygen atoms or with a combination of S and O were unsuccessful. The paper also contained information about the As and Sb speciation. In all the samples, bond distances and coordination numbers for Sb^{III} and As^{III} were constant, whereas for Tl coordination numbers, ranging from 3 to 6, were found. The researchers suggested that Tl^{I} occurred in structural defects in the pyrite that were lacking any long-range order, as there were no spectral features corresponding to coordination shells higher than the first.

Two *mixed micelle CPE LL-MM-CPE procedures* have been developed for the (a) sequential and (b) simultaneous extraction of Tl^{I} and Tl^{III} from groundwater, coal mine and coal fly ash effluents.[174] In the sequential procedure, DTPA was added first to stabilise the unstable Tl^{III} in presence of Tl^{I}. Then, mild surfactants (Aliquat-336/Triton X-114) were added to form cationic mixed micelles that react electrostatically with the stabilised anionic Tl^{III}, which was preconcentrated into the small volume surfactant rich phase, leaving the un-reacted cationic Tl^{I} in the supernatant. After separation (by decanting), HCl was added to the supernatant to form anionic $TlCl_3^{2-}$, which was then preconcentrated by the same procedure. For the simultaneous extraction, the same procedure was followed after the addition of both HCl and DTPA, allowing total Tl to be determined. The viscosity of the mixed micelles rich phase (1.2 mL) was decreased by mixing with 0.8 mL of MeOH containing 2% HNO_3, and the Tl measured by continuum source GFAAS. The injection volume was 20 µL and transversely heated, iridium-coated, pyrolytically coated, platform graphite tubes were used. The possibility of EDTA as a stabilising agent was investigated and a variety of parameters were optimised in a single-cycle alternating variable approach. The LOD was 15 pg mL^{-1} for both procedures, with a linear range of 0.5 – 25 ng mL^{-1} in groundwater samples. The method was validated by interference studies, spike recoveries (at 0.5, 1.0 and 2.0 ng mL^{-1}) from three real samples, and the analysis of CRM NIST1643c (trace elements in water) certified for total Tl of 7.9 ng mL^{-1}. For the CRM, values of Tl^{I} and Tl^{III} of 3.25 ± 0.06 and 4.55 ± 0.05 ng mL^{-1} were obtained by the sequential method, and 7.75 ± 0.07 ng mL^{-1}, by the simultaneous method. Both species were detected in all three samples. The numbers in the presented table of spike recovery values are not calculated correctly according to how the columns are labelled. Calibration was by standard additions.

3.22 Tin

There have been few *reports focusing on Sn speciation* in this review period. The Inorganic Analysis Working Group (IAWG) of the International Consultative Committee for Amount of Substance (CCQM) have evaluated 11 national metrology institutes for measurement of organo-tin in a leather matrix material and other similar organic content materials[175]. The National Institute of Metrology (NIM), China, acted as the coordinating laboratory. The TBT content in leather powder, with a concentration range of 150-600 nmol g^{-1}, was measured using ID-LC-ICP-MS, ID-GC-ICP-MS and LC-ICP-MS. Good agreement was obtained between the laboratories reflecting the maturity of these established measurement protocols. A study of the relationship between urinary TMT and DMT and blood TMT and DMT has been reported[176]. Two tin species, DMT and TMT, in blood collected at different times from three patients contaminated with organotins were measured using HPLC-ICP-MS. Previously published data of urinary DMT and TMT were used for comparison. Both regression analyses and multiple regression analysis with dummy variables were used. The multiple regression analysis showed a significantly positive relationship between urinary TMT and blood TMT, indicating that urinary TMT can be used as an exposure marker for TMT, derived from both external exposure to TMT and conversion of DMT in the human body. The determination of butyltin in seafood by GC-ICP-ID-MS continues to be reported[177]. The working range in this particular study spanned several orders of magnitude from 3.3 - 1010, 2.4 - 785, and 0.3 - 900 ng Sn g^{-1} dry weight for MBT, DBT, and TBT, respectively. The trueness of the method was evaluated by analysing the ERM CRM 477 (Mussel Tissue) and NIES CRM 15 (Scallop). Recoveries were 78 ± 14%, 80 ± 6%, and 88 ± 8% for MBT, DBT, and TBT in ERM CRM 477 and 96 ± 5% for TBT in NIES CRM 15.

3.23 Uranium

The *stability of metaschoepite* (a U^{VI} oxide present in the environment as consequence of U mining wastes) and its impact on U transport and distribution in the environment was evaluated by TEM and bulk micro and nanofocused- XAS and XRF analysis.[178] For this purpose, sediments were doped with discrete horizons of metaschoepite particles. Afterwards, the metaschoepite was subjected to ground-water flowing under oxic and anoxic conditions for periods of up to 12 months. During this period effluent samples were periodically collected and monitored for changes in pH and concentration of Fe, Mn, NO_3^-, SO_4^{2-} and U. In the column exposed to oxic conditions no changes in Fe, Mn, NO_3^- and SO_4^{2-} were detected suggesting that the system remains aerobic during the entire reaction period. However, UO_2^{2+} was identified in the effluent after 6 months of reaction which indicates a significant U^{VI} dissolution transport. In the anoxic columns significant amounts of UO_2-containing colloids were detected as suggested by TEM measurements. Analysis by XAS confirmed the presence of U^{VI} and biogenic UO_2. The authors highlight the importance of U^{IV} colloid production from U^{VI} solids under reducing conditions. This finding is of special relevance to correctly manage sites contaminated with U wastes as colloids are known to enhance contamination transport through the environment.

3.24 Zinc

In *farmed fish, Zn deficiency* has been associated with impaired growth, skeletal abnormalities and reduced activity of various Zn metalloenzymes. The composition of salmon feed has changed from the use of mainly marine fish (fish meal and fish oil) to plant-based ingredients.

This change in composition has resulted in the need to add Zn supplements to the feed to ensure adequate Zn levels in the fish. In this sense, several Atlantic salmon feeds were characterized in terms of Zn species by employing a SEC-ICP-MS methodology.[179] Special attention was paid on selecting the best Zn extraction procedure by using a fractional factorial design. Feed samples were treated with a mixture of AcH:Tris HCl as an extraction solution at different concentration levels (10, 100 mmol L^{-1}), pH values (6.5, 8.5), temperatures (20 and 4 °C), treatment time (1 and 24h) and with and without adding 4% SDS. The highest Zn recovery (98 ± 6%) was obtained by selecting 100 mmol L^{-1} Tris-HCl, pH =8.5 at 4 °C and 24 h as optimal conditions. For the separation and detection of Zn species, a TSKgelG3000WxL (30cm x 7.8 mm; 5µm) column coupled to the ICP-MS was used. A mixture composed of 50 mmol L^{-1} TRIS HCl and 3% MeOH (pH 7.5) flowing at 0.7mL min-^{1} was employed as mobile phase. The detected Zn-containing peaks were associated to high molecular weight (>600kDa), medium molecular weight (32 to 72kDa) and low molecular weight (17 to 1.36 kDa) compounds, with the latter as the predominant Zn species in the salmon feed accounting for 84-95% of total Zn.

The increasing use of ZnO NPs in many consumer products has made them a potential source of Zn in the environment, with one of the main pathways for ZnO NPs for this being wastewater treatment plants. Two papers report on the fate and metabolisms of ZnO NPs in environmental systems. The first paper describes the application of hard X-ray fluorescence microscopy (XFM) and SEM to characterize and evaluate the morphological transformations of ZnO NPs in a simulated sludge.[180] Synthetic ZnO nanorods (725 nm length and 140 nm of diameter) were incubated for 1 and 3 h in the presence of 10 mg L^{-1} humic acid as a relevant aqueous component of wastewater sludge (which does seem a bit questionable as humic acids are derived from soils and thus may not be representative of the organic component of real wastewater sludge). Results provided by the different techniques evidenced a significant decrease of ZnO NP concentration with time and the occurrence of ZnS as the predominant Zn compound. The presence of diffuse nanoparticles of ZnS, $Zn_3(PO_4)_2$ and Zn adsorbed to Fe-oxyhydroxides were also imaged. These types of studies are of special relevance to assess correctly the impact of NPs in the environment. The second paper evaluates the fate of ZnO NPs in non-accumulator plants.[181] *Lactuca sativa* was hydroponically cultivated and exposed to Zn supplied in the form of ZnO NPs and $ZnCl_2$ at a concentration level of 5 mg L^{-1} for 7 days. The collected plants were subsequently divided into roots, leaves and stems before analysis. A wide variety of analytical techniques were used: ICP-MS (total Zn), spICPMS (ZnO NPs dissolution), SEC-ICP-MS and HILIC-ICP-MS (Zn speciation) and HILIC-ES-FT-Orbitrap-MS and

HILIC-QTOF-MS/MS (identification of extracted Zn compounds). The ICP-MS measurements, after acid digestion of the plant tissues, provided equal Zn content in treated plants regardless the chemical form of Zn supplemented. This fact was explained by the authors as a consequence of the rapid dissolution of ZnO NPs in the growing media. The spICP-MS measurements corroborated the importance of ZnO NP dissolution as around 70% of the added NPs disappeared after 4 minutes of exposure. Results evidenced that plants accumulated Zn mainly in the form of dissolved Zn as the uptake of ZnO NPs was likely to be negligible due to their dissolution. In addition, Zn compounds were quantitatively extracted from plant tissues by employing a sequential extraction consisted of two steps: 1) 10 mmol L^{-1} NH_4Ac pH 6.8, and 2) 4% peptinase plus 4% cellulose at pH 4.5. A 2D chromatographic approach (2D-SEC-HILIC-ICPMS) was applied to the resulting extracts. Only those fractions obtained by applying NH_4Ac as extracting agent provided significant results. In order to identify the nature of the Zn –complexes present in the plant tissues, the fraction was subsequently analysed by HILIC-tandem mass spectrometry. Nicotianamine was found to be the major ligand binding Zn.

An interesting approach based on the use of *XANES spectroscopy in tomography mode* has been developed to evaluate the effect of the infection with Turnip Yellow Mosaic Virus (TYMW) on Zn speciation in leaves of the non-accumulator plant *Noccaea ochroleucum* grown in presence of 100 mmol L^{-1} Zn.[182] The method allowed the authors to perform Zn speciation in intact frozen plant tissues with a spatial resolution lower than 5 µm. The ability of the technique to measuring metal content and chemical species in frozen samples offers the important advantage of decreasing the formation of artifacts during sample preparation which can be a source of error. Moreover, tomography allows the making of measurements in the interior of samples without the need of cutting to unveil the surface. Unfortunately, the size of the sample is a limiting factor as smaller samples requires a higher resolution. In the current work a spatial resolution of 5 µm was low enough to resolve all leaf tissues and cells. The presence of the virus did not affect the metal distribution and speciation in leaf tissues where Zn appeared mainly as Zn-histidine and Zn-phosphate. The most significant difference in the virus treated *N. ochroleucum* was the presence of Zn spots similar to Zn silicate.

4 Biomolecular Speciation Analysis

Two distinctly different *reviews covering the instrumentation and methodology used for biomolecular speciation* have been published. The use of atomic spectrometry and atomic mass spectrometry in this subject area has been reviewed and covers 142 papers in the period 2013 to 2018, the majority originating from Chinese scientists, highlighting the recent excellent research being published from China.[183] The focus is similar in coverage to this section of ASU in reviewing studies related to the development of methods for the measurement of toxicologically and physiologically important species in biological samples, elements, small molecules, proteins, nucleic acids and cells. The narrative makes the point that whilst MALDI-MS or ES-MS and their hyphenation with separation techniques are widely used in omics-related research, in contrast, methods based on atomic spectrometry are preferred when quantitation is required. Limitations in molecular MS become evident when absolute quantitation and method validation are required, due to the lack of available standards and CRMs. The review covers new applications in various non-traditional omics-related areas including: quantification and imaging methodologies for trace elements, engineered nanoparticles and metal-based drugs in biological samples, to elucidate their stability, reactivity, toxicity and distribution in tissues, cells and organs; quantitation of biomolecules via the naturally occurring heteroatoms (S, P, and Se) by the combined use of orthogonal separation methods; metal tagging / labelling for the sensitive determination of a variety of targets (small molecules, peptides, nucleic acids, proteins and even cells) with metal-containing compounds, bifunctional chelating reagents, or nanoparticles; and label-free schemes for detection of biomolecules. A second instrumental review, specifically on analytical and biophysical methods for the study of ligand-protein interactions, but with a focus more on the biophysical methods used for metal-protein interactions, includes 213 references covering the period between 1997 and 2019.[184] The main application themes covered in the review include: micro- and nanoscale analysis; kinetics; structural analysis of biomacromolecular complexes; metal-protein stoichiometry; and enthalpy of biological systems. The methods reviewed included: ES-MS/MS, X-ray crystallography, NMR-spectroscopy, several spectrophotometric techniques, calorimetry and other thermal methods, each being complementary with specific strengths and limitations. All of the analytical platforms described were for use on purified proteins, rather than crude extracts and for this reason no separation based methods coupled to ICP-MS or other chromatographic detector were reviewed. The use of LA-ICP-MS is briefly covered for the imaging of tissue sections and electrophoresis gels, but not in any depth.

4.1 Direct biomolecular analysis

Two unrelated *studies on the speciation of trace metals and Pd-nanoparticles in plants have highlighted improvements in chromatographic resolution, elemental sensitivity and the use of* *orthogonal detection methods*, which make it possible to investigate trace metal binding to proteins and pigments at environmentally relevant concentrations and the investigation of ligands generated in response to Pd exposure. Kupper *et al.*[185] have designed a sophisticated HPLC-SF-ICP-MS system with a metal-free flow path and a switching system so that high concentration calibration standards do not come into contact with the chromatographic columns used, which can lead to number of problems due to metals being absorbed onto the column packing material. In unison, they also purified the buffers, using Chelex-100, to reduce the metal content and in this way were able to achieve very low baseline signals. Unfortunately, for some reason no actual LOD values were given in the paper, however the response for 1.0 nmol L^{-1} La was quoted as 7,500 cps in medium resolution and the chromatographic baseline signal for elements such as Cr, Cu and Fe were low. To overcome the common problem in metalloprotein analysis of measuring high signal elements such as Mg, P and S simultaneously with very low signals from the analytes of interest, the SF-ICP-MS instrument was equipped with an additional Faraday cup as a detector for high count rate elements. The use of SF-ICP-MS hardware is not normally suitable for speciation studies because of the low scanning speed, compared to quadrupole instruments, when carrying out multi-elemental transient signal acquisition, but unfortunately this aspect of the work was not discussed. The instrument was operated in medium resolution mode, which can reduce the signal due to attenuation of the ion beam. However, improvements in sensitivity were facilitated by replacing the standard spray chamber with a desolvating nebuliser (Apex Q) and the use of a jet interface, which is essentially a strong interface vacuum pump behind the sample introduction system. By using different combinations of 2 types of SEC columns, Superose Increase and Superdex Increase (10 x 300 mm, 8.6 μm), containing cross-linked agarose or dextran-agarose respectively, reasonably good resolution for SEC was achieved. The proteins were eluted isocratically using a mobile phase containing ammonium acetate (150 m mol L^{-1}) and a surfactant, dodecylmaltoside (0.2 m mol L^{-1}). The system was applied to a number of plant studies looking at protein-metal binding at low and ambient concentrations of Cd, Cr, Cu and La, however none of the proteins involved were characterised or any attempt given at their identification which was a significant drawback of the report, presumably this will be the focus of subsequent papers. Methods for the speciation of technologically critical elements, such as Pd, are needed to address their growing environmental impact, but also to aid their recovery and recycling. The group in Pau have developed a novel 2D HPLC system coupled in parallel to ICP-MS and ES-MS^n (Orbitrap) to investigate the uptake and metabolism of Pd nanoparticles in plants grown hydroponically.[186] Initially, SEC coupled to ICP-MS was used to show a number of Pd-containing biomolecules, the most abundant with a mass <0.2 kDa was shown to form metabolically rather than coincidentally in the plant/hydroponic system. This compound was isolated by fraction collection and subjected to hydrophilic interaction chromatography (HILIC) coupled to ES-MS^n for identification. This chromatographic approach was used because HILIC is suitable for the separation of small polar compounds such as amino acids, peptides and proteins and the eluent is compatible with the ES source. For the SEC separations either a Superdex 200 (separation range: 10 – 600 kDa) or a Superdex Peptide 300 (separation range: 0.1 – 7 kDa) column was used with isocratic elution with ammonium acetate (100 m mol L^{-1}, pH 7.5) flow rate 0.7 mL min^{-1} as the eluent. For the ES measurements either a SeQuant Zic-cHILIC or a Kinetex HILIC column was used, both eluted with some form of ammonium acetate pH 5.5 buffer - acetonitrile gradient at a flow rate of 0.2 or 0.5 mL min^{-1} respectively. The metabolically formed Pd-complex was identified by ES-MS^n analysis by scanning for the characteristic Pd-isotopic pattern. Initially two candidate compounds with m/z 408.038 and 415.034 were investigated by fragmentation analysis using the MS^n mode and also by coupling the HILIC separation to an ion-mobility MS, which can provide separation of ions with similar m/z values by their cross-sectional area when passing through a buffer gas. The final identification of the metabolically formed complex was Pd bound to two histidine molecules. This excellent piece of detective work illustrates the complexity of speciating unknown metal binding biomolecules in real samples and highlights the inadequacy of the former work using SF-ICP-MS detection alone to investigate trace metal binding in plants.

An interesting *device for coupling GE on-line with ICP-MS detection* has used 3D printing to produce the major components.[187] One of the main difficulties in the measurement of metalloproteins is their separation as intact proteins without the loss of the metal co-factor, with a suitable degree of peak shape and resolution, such that there is confidence that co-elution has not occurred, but also so that the peaks have good S/N ratios. Most modes of HPLC chromatography are able to do this to some degree, but GE offers excellent performance for the high resolution separation of proteins. The main drawback, as has been noted previously, is how to couple the GE column to the detector without loss of separation power. In this paper the authors have designed and built a GE device consisting of three main components: a horizontal gel separation tube; gel electrophoresis tank to maintain electrical conductivity; and an elution chamber to couple the separation to the ICP-MS/MS detector. The major components were made using 3D printers and printing materials that are common on the market and the authors have generously made available the program and specifications as part of the supplementary material for the paper. A gel tank was required to allow sufficient cooling of the gel for high separation voltages to be used. The performance of the system was initially tested off-line in comparison to a conventional slab gel, with the same gel composition and length, using a commercially available Precision Plus Protein Dual Colour protein standard. The resolution between the 50 / 37 kDa and 20 / 15 kDa protein bands obtained by the 3D desk-top printer constructed GE device was 1.53 and 1.56. The same protein bands separated by slab GE device were 1.61 and 1.58, respectively. The results indicate that at middle and low molecular range, the separation efficiency of the GE device was comparable to the commonly used slab GE separation system. The device hyphenated to the ICP-MS/MS detector was first tested using iodinated Ribonuclease A and the results showed an integratable peak, but which was broader using a printed device compared to a commercial 3D printer. This was shown by SEM analysis of the inner separation tube to be due to surface roughness. The system was tested further using mouse blood spiked with $HgCl_2$ (10 mg L^{-1}) and several Hg-binding proteins including: haemoglobin; glutathione peroxidase 3, apolipoproteins AI and A4, and albumin/selenoprotein-P were separated. Unfortunately, the concentrations were only sufficient for the haemoglobin-Hg adduct to be above the LOD. Clearly this is a useful device and with further refinement will be a useful tool for interfacing GE and ICP-MS.

4.2 Tagging methods for macromolecular analysis

A wide ranging review of *quantitative laser ablation ICP-MS methods for mapping specific proteins in biological tissues using exogenous labels* has been published in a special collection of papers on the subject of Elemental and Molecular Imaging by LA-ICP-MS in the journal Analytical and Bioanalytical Chemistry.[188] Historically, the initial studies using LA-ICP-MS for the analysis of tissue samples focused mainly on determining the spatial distribution of metals, showing the co-distribution of elements, but providing no molecular information related to the binding partner. Recent work, as detailed by the 69 papers described in this review, have used antibodies labelled with one or more metal atoms to provide some specificity and molecular information when using this technique. This review goes further in highlighting recent work providing not only molecular information but also demonstrating the quantitative, rather than just qualitative, data that this methodology can provide. Critical requirements to obtain absolute quantitative mapping of specific proteins by LA-ICP-MS are highlighted, using illustrative examples of the advances made so far with LA-ICP-MS. In most cases the immunohistochemistry protocols commonly used in diagnostic histology laboratories have been adapted for use, sometimes in parallel to the usual fluorescence microscopy methods used by histopathologists. The review covers all the practical aspects of the procedure including: the critical requirements for absolute quantitation of proteins; the labels that have been developed for mapping; and the labels containing single or multiple elements or isotopes. Some of the more interesting applications cover: the measurement of specific proteins in retinal tissue; epidermal growth factors in breast tissues related to cancer studies; and the measurement of dopamine synthesising enzymes in mouse brain tissue. With the emergence of truly simultaneous detection instrumentation for elemental/isotopic analysis, such as ICP-TOF-MS, coupled to improved laser ablation cells and with better and more consistent labelling reagents, this area will no doubt see a significant expansion as this hardware is more fully adopted. That said, one caveat applies as always relating to the pre-analytical sample preparation steps to not only take a representative sample, but also prepare it for analysis. To do this without changes in protein conformation is still a significant challenge to understand and overcome.

The *development of new labels with greater functionality or higher metal loading are widely applicable to the improvement of assays for the measurement of proteins and other biomolecules* in biological samples by elemental MS. By exploiting the presence of hexa-histidine tags that are often present for other practical reasons in recombinant proteins and which have a high affinity for metal ions, it was possible to label recombinant proteins without changing the protein surface, which can lead to changes in the biophysical properties of the protein.[189] Whilst many divalent metals can bind to poly-histidine sequences irreversibly this method used the oxidation of imidazole-bound Ru^{2+} to dramatically increase the binding strength. The method was developed using a synthetically produced model peptide YPDFEDYWMKHHHHHH and mild oxidation of the Ru^{2+} ion under ambient conditions, which avoided any damage to the peptide during the process. The resulting peptide-Ru^{2+} complex was stable, with the single hexa-histidine segment capable of accommodating up to three metal ions, which increased the metal loading and therefore detectability of the peptide. Structural characterisation of the labelled peptide complexes was confirmed using FT-ICR-MS with a standard ES source. The stability of the binding was also assessed using different competitor ligands, which were unable to remove the Ru ion from the tag. The methodology was applied to labelling an 80 kDa recombinant form of the Fe-containing protein transferrin, the metal label did not interfere with receptor binding, while allowing the protein to be readily detected in serum at sub-nmol L^{-1} concentrations by ICP-MS. This approach clearly has application in studies related to protein-protein interactions and investigations into the biochemical functioning of a wide range of model recombinant protein systems. The use of ICP-MS detection for the measurement of biomolecules tagged with metals has some distinct advantages, such as a greater degree of multiplexing being possible because of resolution of MS methods compared to conventional spectroscopic methods, which are limited by band broadening. However, the sensitivity using inorganic MS is not significantly greater than conventional fluorescence-based methods. One very promising approach to improving sensitivity is by designing elemental tags that are able to accommodate a large number of metal atoms.[190] Nanoparticles and QDs have been proposed as a way of providing greater sensitivity but their effectiveness is affected by non-specific absorption and non-uniform particle sizes, which can greatly reduce the S/N ratio. The addition of a greater number of metal atoms can be facilitated by replacing the normal nucleotides in DNA with the alkyne nucleotides, to obtain alkyne-DNA scaffolds, using PCR. Primers with reactive groups for tagging the target biomolecules were also incorporated into the synthesized DNA scaffolds. The metal-DOTA-N3 complex reacted with the alkynyl groups in the DNA strands by a click reaction. The elemental tag was then modified with a biotin group and streptavidin as a cross-linker, to allow conjugation of the elemental tag with the required antibody. This approach was trialled on the measurement of cancer biomarkers such as carcinoembryonic antigen and alpha-fetoprotein and showed an increase in sensitivity by two orders of magnitude compared to the same tag containing a single metal ion. Application areas for this methodology would be in clinical diagnosis, cancer treatment and mass cytometry of cells.

Recent *methodological improvements to labelling protocols or multiplexing of assays has involved the use of solid surfaces to isolate proteins prior to ICP-MS measurement*. Particle imprinted polymer technology has been developed for use with QD (CdS) based immunoassays and LA-ICP-MS for the measurement of IgG in complex mixtures containing proteins in the size range 10 - 250 kDa.[191] The principle behind the use of molecularly imprinted technology (MIP) is based on the formation of an analyte recognition surface that is created after polymerization of a mixture of analyte and suitable monomer, the main advantage being its wide applicability compared to the use of immunochemical methods, where an antibody has to be available or generated using animal hosts. If antibodies are not available then MIPs can be employed for a broad range of analytes, from ions to microorganisms and take advantage of various surface arrangements including: well-plates; microscopic slides; mass spectrometric targets; and nanoparticle surfaces. However, in the current work two approaches utilising antibodies were used to create the template to form the MIP. In the first concept the antigen was used as the template and the MIP selectively isolated it from the sample and subsequently the MIP surface was overlaid by the QD-antibody conjugate. In the second approach, the whole QD-antibody-antigen complex was imprinted and during the analysis this complex was extracted from the sample by the MIP. In both cases a non-covalent imprinting approach using oxidative polymerization of dopamine created the self-assembly monolayer of the MIP. The LA-ICP-MS parameters were optimised for the detection of the CdS QD via the ^{111}Cd signal. Whilst the concept of using MIP appears enticing, it is clear that from a practical perspective it suffers significant matrix effects: in the first approach the antigen orientation was not ideal for binding the antibody-QD complex so the Cd signal was too low; and in the second approach, there was significant non-specific binding of the QD-antibody-antigen complex to the surface resulting in a poor S/N ratio. The situation deteriorated further when a protein matrix was included in the assay, suggesting that this promising approach needs refinement before being applied to specific investigations in real world samples. In a preliminary study on the simultaneous measurement of albumin and IgG in whole blood, plasma and dried blood spots, an antibody labelling method involving Eu and nanogold was compared to commercially available ELISA assays.[192] The advantage of this approach is that simultaneous measurement of different proteins can be facilitated by using different metal labels, which is a distinct advantage over current ELISA methods which are usually designed for a single analyte. However, in the current study the calibration curves for the measurement of IgG by both methods were very poor indicating some underlying issue with both assays. It should also be noted that ELISA methods have a number of significant disadvantages such as the Hook effect, cross-reactivity of antigens-antibodies, poor linear ranges and significant practical drawbacks, such as limited sample numbers and batch analysis. It is for this reason that many clinical laboratories are now replacing these methods with LC-MS/MS assays which overcome many of these disadvantages. It is difficult to see how replacing the conventional detector by ICP-MS would offer much in the way of overcoming these drawbacks. Interestingly, the work also investigated the use of finger-prick sampling and dried blood spot analysis, both controversial pre-analytical approaches in clinical chemistry. In general, even if it is possible to obtain enough of the analyte, smaller samples can be poorly representative of the circulating biomolecules being measured. When developing new analytical strategies it has to be remembered that whilst analytical scientists may regard good accuracy and high precision of a method as the holy grail, clinicians want to be sure that the concentration measured in the sample, reflects the level circulating in the patient.

5 Abbreviations used in this update

AAS	atomic absorption spectrometry
AB	arsenobetaine
AC	arsenocholine
AE	anion exchange
AEC	anion exchange chromatography
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
AOAC	Association of Official Agricultural Chemists
APDC	ammonium pyrrolidine dithiocarbamate
AsHCs	arsenohydrocarbons
ASU	Atomic Spectrometry Update
BARGE	Bioaccessibility Research Group of Europe
BCR	Community Bureau of Reference
BW	body weight
CCA	chromated copper arsenate
CCQM	Consultative Committee for Amount of Substance
CE	capillary electrophoresis
CEC	cation-exchange chromatography
CIC	conjoint liquid chromatography
cIEF	capillary isoelectric focusing
CPE	cloud point extraction
CRM	certified reference material
CV	cold vapour
CZE	capillary zone electrophoresis
Da	Dalton
DCM	dichloromethane
DLLME	dispersive liquid-liquid microextraction
DLS	dynamic light scattering
DMA	dimethylarsenic
DMAE	dimethylarsinoyl ethanol
DMAP	dimethylarsinoyl propionate
DMT	dimethyltin
DNA	deoxyribonucleic acid
DRC	dynamic reaction cell
DTPA	diethylenetriaminepentaacetic acid
Dw	dry weight
EDTA	ethylenediaminetetraacetic acid
EDX	energy dispersive X-ray fluorescence

ELISA	enzyme-linked immunosorbent assay
ES	electrospray
ETA	electrothermal atomisation
EtHg	ethylmercury
EU	European Union
EXAFS	extended X-ray absorption fine structure
FAO	Food and Agriculture Organisation
FC	frontal chromatography
FDA	Food and Drink Organisation
FFF	field flow fractionation
FT	Fourier transform
FTIR	Fourier transform infrared
GC	gas chromatography
GE	gel electrophoresis
GPC	gel permeation chromatography
HAS	human serum albumin
HG	hydride generation
HILIC	hydrophilic interaction liquid chromatography
HPLC	high performance liquid chromatography
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	inductively coupled plasma optical emission spectrometry
ICR	ion cyclotron resonance
ID	isotope dilution
IDA	isotope dilution analysis
LA	laser ablation
LC	liquid chromatography
LDI	laser desorption ionisation
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
LPME	liquid phase microextraction
MAE	microwave assisted extraction
MALDI	matrix-assisted laser desorption ionization
MALS	multiangle light scattering
MBT	monobutyltin
MC	multicollector
ME	microextraction

MeHg	methylmercury
MeOH	methanol
MeSeCys	methylselenocysteine
MMA	monomethylarsenic
MRI	magnetic resonance imaging
MS	mass spectrometry
MT	metallothionein
NAA	neutron activation analysis
Nd:YAG	neodymium doped:yttrium aluminum garnet
NIES	National Institute for Environmental Studies
NIST	National Institute of Standards and Technology
NMG	N-methylglucamine
NMIJ	National Measurement Institute of Japan
NMR	nuclear magnetic resonance
NP	nanoparticle
NRCC	National Research Council of Canada
ODS	octadecylsilane
PAGE	polyacrylamide gel electrophoresis
PET	polyethylene terephthalate
PhHg	phenylmercury
PIXE	particle-induced X-ray emission
PLS	partial least squares
PTFE	poly(tetrafluoroethylene)
PV	photochemical vapour
QD	quantum dot
RM	reference material
ROS	reactive oxygen species
RP	reverse phase
RSD	relative standard deviation
SDS	sodium dodecylsulfate
SeAlb	selenoalbumin
SEC	size exclusion chromatography
SelP	selenoprotein
SEM	scanning electron microscopy
SeMet	selenomethionein
SF	sector field
SIMS	secondary ion mass spectrometry
sNP	single nanoparticle

SOD1	superoxide dismutase 1
SPE	solid phase extraction
SPME	solid phase microextraction
SRM	standard reference material
SR-XRF	synchrotron radiation X-ray fluorescence
ssIDA	species specific isotope dilution analysis
ssIDMS	species specific isotope dilution mass spectrometry
TBAH	tetrabutylammoniumhydroxide
TBT	tributyltin
TEM	transmission electron microscopy
TIMS	thermal ionization mass spectrometry
TMA	trimethylarsenic
TMAH	tetramethylammonium hydroxide
TMAO	trimethylarsine oxide
TMAP	trimethylarsoniopropionate
TMOS	tetramethoxysilane
TMT	trimethyltin
TOF	time of flight
TRIS	tris(hydroxymethyl)aminomethane
UAE	ultrasound assisted extraction
US EPA	United States Environmental Protection Agency
UV	ultraviolet
VG	vapour generation
WD XRF	wavelength dispersive X-ray fluorescence
WHO	World Health Organisation
XANES	X-ray absorption near-edge structure
XAS	X-ray absorption spectroscopy
XPS	X-ray photoelectron spectroscopy
XRF	X-ray fluorescence

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